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News

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ORTHO

EMILIO PEPE MICHAEL MEINECKE
1869–1957

W. W. Wagener, Carl Hartley, and J. S. Boyce

By the death of Dr. E. P. Meinecke in San Francisco on February 10, 1957, at the age of 87, forest pathology lost one of its outstanding men of all time.

Dr. Meinecke was born in Alameda, California, on July 26, 1869. His German parents were early residents in California, his maternal grandfather having established the first bookshop in newly burgeoned San Francisco about 1850; his father had an importing business there several years later, afterward serving as German consul-general at the port. Botanical science was already firmly established as a pursuit in Meinecke's lineage by his distinguished maternal great-uncle, Matthias J. Schleiden, Professor of Botany at Jena, to whom we owe the development of the cell theory in plants. His example undoubtedly influenced Meinecke in a choice of field of university study. Invalidism in the family caused a move to Germany for a time while he was still a boy, and he remained to obtain almost all of his education there, attending the universities of Freiburg, Leipzig, and Bonn and completing the requirements for a Ph.D. degree at Heidelberg in 1893 with a dissertation on aerial roots in the orchid family.

EMILIO PEPE MICHAEL MEINECKE
1869–1957

Meinecke remained as an assistant in the Botanical Institute at Heidelberg for two years. Then an interest in wine yeasts drew him to Hansen's laboratory in Copenhagen, at that time regarded as perhaps the foremost center for yeast investigations in the world. His work in this field culminated in the publication, with Edmond Kayser in 1898, of the manual "*Die Hefe.*" The same year he became assistant to that pioneer forest pathologist Robert Hartig at the Forest Academy of the University of Munich, remaining there for a year following the latter's death in 1901. The association with this talented and indefatigable scientist profoundly influenced Meinecke and really determined his future. At that time, however, openings in the forest disease field were practically nonexistent. In the interval, with the encouragement of Warming, he turned to the translation of the Warming-Johannsen textbook of general botany from its original Danish into the more widely read German. In 1907 he accepted a call as Assistant Professor of Botany in the University de La Plata in the Argentine. Here he revelled in the opportunity for trips with a geological colleague into the southern Andes; however, the ultraconservative academic climate then prevailing at the institution presented limited opportunities otherwise, and in 1909 he returned to his native California. The following year he was appointed to the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, and was assigned as consulting pathologist for District 5 of the U. S. Forest Service, with headquarters in San Francisco. He remained in this position until 1928, when his responsibilities were made nationwide as consulting pathologist for the National Park Service. This post he held until his retirement in 1939.

Forest pathology, along with forestry, was still in its formative period at the time that Meinecke assumed his duties in San Francisco. Foresters in the West were struggling with the problems of placing the national forests under administration and in controlling fires with the means at hand; they knew practically nothing of forest pests and diseases. The handful of technical workers in the forest disease field in the United States had been able to make only a start in exploration of disease problems in the nation's forests. Much of their attention had, by necessity, been shifted to those offered by 2 introduced diseases, chestnut blight and white pine blister rust, the latter then only recently discovered on the Atlantic seaboard. Meinecke recognized the immediacy of the need for a knowledge of the disease organisms active in his territory and even before his appointment had begun the series of reconnaissance trips by horseback through the forests of California that provided the background for the field manual "*Forest Tree Diseases Common in California and Nevada,*" published in 1914, the first of its kind in the United States. He was looking beyond this reconnaissance activity, however, and in 1911 began the studies that were to result in the bulletin "*Forest Pathology in Forest Regulation,*" a classic not only in forest pathology but also

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in forestry. In this bulletin, which appeared in 1916, he went far ahead of previous thinking, which was concerned with individual organisms and their effect on individual trees, by developing the concept of cumulative risk of loss with age and of the effects of disease as applied to forest stands rather than to trees as individuals. He demonstrated that, if maximum yields were to be obtained, foresters must take the loss factor from diseases into account in forest management, scheduling cuts at an age that would forestall heavy losses from heart rots. These concepts have had a far-reaching effect on research and forest practice in the United States and Canada.

Before this bulletin appeared, Meinecke was instrumental in securing the adoption of the so-called sanitation clause into Forest Service timber sale contracts. By its provisions, contractors were required to cut snags and cull trees whether utilizable or not. Western foresters, acutely aware of the potentialities of snags as fire spreaders, were quick to appreciate the hazard-reduction features of this clause, and they could also understand the advantages of ridding the forest of unmerchantable trees to make way for new growth; Meinecke, however, was looking far ahead to the sanitary effect on the coming forest of the destruction of sources for heart-rotting fungi. Even with killing diseases, Meinecke never lost sight of the forest approach. At a time when others were assessing damage in terms of proportions of plants attacked or killed, he was pointing out that in forests the real measure of damage was the effect on stocking and thus on future yield from the attacked stand.

In still other aspects Meinecke broke new ground. He was the first in America to demonstrate direct tree-to-tree infection by a coniferous stem rust fungus, without the necessity for an intermediate host; a "repeating pine rust," to use his term. In the late 1920's, he became concerned over the dangers to outstanding examples of tree growth, such as the picturesque native Monterey cypress groves and the most accessible giant coast redwoods, from the effects of heavy and unrestricted human use. Up until that time all effort had been directed toward making such objects of natural beauty and grandeur available for public enjoyment without realization that the public, without guidance, might ultimately destroy the very values for which such areas had been reserved. In a series of reports and publications, he not only called attention to these dangers but also to the necessity for recreational planning that takes into account both native and human needs and values.

Meinecke found little interest in the routine development or proving of facts. Statistical analysis left him cold. It was the fresh idea, the new approach, that attracted him; if the evidence seemed biologically sound and numerically convincing, he was willing to accept it. He was not a prolific writer but anything that he wrote was worth reading, not only for the subject presented, but also for the manner of presentation. Few could write with greater clarity, logic, and preciseness of meaning.

In many other respects Meinecke was a man of exceptional talents and wide interests. He painted competently in oils and water colors. He played the piano extremely well. He was an accomplished skier and figure skater, a connoisseur of foods, and a superb cook, with a knowledge of recipes from many lands. In later years he became an enthusiastic gardener and delighted in sharing his gardening triumphs with others. He read much and widely. To a solid classical foundation in Latin and Greek, he added an exceptional command of five modern languages and an adequate reading knowledge of several more, a facility deeply appreciated by visiting foreign scientists. He was a member of numerous professional and scientific societies and took an especial interest in the California Academy of Sciences, serving on the Council of the institution and for two terms as Vice President. At home or at the University Club he was the perfect host. Many an oldtime forester can recall with nostalgia the pleasant gatherings that for a number of years were an annual event at his Jackson Street home.

Dr. Meinecke was a wonderful teacher, and his influence on men, who in their younger days served as his assistants, has been profound. No matter how busy he was, time was always found to instruct and encourage young men. Nor was his help limited to them. His counsel was frequently sought and cheerfully given on many problems. More than one busy forester, harassed by an approaching deadline on an important resource report, has benefitted from Meinecke's keen analysis in rearranging the preliminary draft to form a logical whole. When, in the mid thirties, new ground was broken in fire guard instruction, it was Meinecke's accompanying sketches that conveyed an understanding of the control problems presented in the new manual. He was much interested in broader education at the ranger level and for many years took an active part in instruction at Forest Service ranger training camps.

One to whom many owe much has passed from among us but his imprint will long remain.

PRODUCTION OF PECTINASE AND GROWTH-PROMOTING SUBSTANCE BY CERATOCYSTIS FAGACEARUM [1]

Charles L. Fergus and David C. Wharton [2]

SUMMARY

In liquid culture, *Ceratocystis fagacearum* (Bretz) Hunt produced no ethylene but did produce a growth-promoting substance that caused inward curvature of the split stems of pea seedlings. Metabolic fluids contained no pectin methylesterase or polygalacturonase. Pectin depolymerase, as determined by viscosity changes of pectin solutions, was produced by the fungus *in vitro*.

The production of pectic enzymes by wilt-inducing fungi has been demonstrated for *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. (3, 8), *Verticillium albo-atrum* Reinke & Berth. (6), and *Ceratocystis ulmi* (Buism.) C. Moreau (1). The production of these enzymes and of growth-promoting substances has been suggested to be of considerable importance in the disease complex (1), especially in gum and tylosis formation. Gums and tyloses have been observed in northern pin and red oaks inoculated with *Ceratocystis fagacearum* (Bretz) Hunt (2). Some of the foliar symptoms of oak wilt, including downward bending of leaves and premature leaf fall, are similar to those that develop on plants exposed to ethylene.

Data on whether or not pectinase and growth-promoting substances are produced by other wilt-inducing pathogens would aid in determining if these metabolites are important factors in the physiology of the wilt mechanism. Therefore, their production by *C. fagacearum*, the oak wilt pathogen, was investigated. In addition, tests were made to determine whether or not ethylene was produced.

MATERIALS AND METHODS.—*Ceratocystis fagacearum* was grown in stationary culture in 50 ml of liquid media in cotton-stoppered 250-ml Erlenmeyer flasks. Two media, each with and without added pectin, were used. They were Medium A, a modified Richard's solution containing 1 g KNO_3, 2.5 g KH_2PO_4, 2.5 g $MgSO_4 \cdot 7H_2O$, 0.02 g $FeCl_3$, and 5 g sucrose per liter, and Medium B, a modified Czapek's solution containing 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $Fe_2(SO_4)_3$, 2 g yeast extract, and 40 g glucose per liter. Pectin was added at a rate of 5 g per liter. Medium A was adjusted to pH 4.5 and Medium B to pH 7.3. The media were autoclaved for 20 minutes at a pressure of 12 lb. sq. in.

Mycelial transfers were made with 13-mm discs cut with a cork borer from a mat of mycelium grown in liquid culture. Conidial transfers were made by pipetting 1 ml of spore suspension containing approximately 200,000 spores per ml into each flask. Cultures were incubated in the dark at 25°C for 14 days.

In enzyme assays, the fungus was removed from the culture solution by filtration through filter paper. The solutions were analyzed immediately or stored at −10°C until required. Pectin methyl esterase was determined by the alkali titration method (10); polygalacturonase by the hypoiodite method (4); and pectin depolymerase by changes in viscosity (1, 7), slightly modified as follows. Fifty ml of 0.5 per cent pectin[3] solution was mixed thoroughly with 10 ml 0.5 M sodium acetate (pH 4.5) and 5 ml fungus filtrate in a 250-ml Erlenmeyer flask, which was then stoppered with a cork and incubated at 31°C for 10 hours. Heated fungus filtrate samples and unseeded media were also tested. Flow time of each was determined by means of an Ostwald viscosimeter in a water bath at 31°C.

The presence of growth-promoting substances in the fungus filtrate was determined by the pea curvature test (9). For this test, Glacier peas were germinated on moist filter paper, then placed in petri dishes containing 10 ml saturated $CaSO_4$, and incubated in the dark. When they had attained a length of 10–12 cm, the tops were cut off 5 mm below the terminal bud. The stem was then split for a distance of 3 cm by means of a razor blade. The split section was then cut off a few mm below the split and washed in distilled water for 1 hour. Twenty-ml portions of the fungus filtrates, adjusted to pH 6, were poured into petri dishes. Eight split sections were then floated in each dish and left in the dark for 6–24 hrs.

The ethylene-production assay method of Pratt and Biale (5) was employed in its entirety, except that peas of the Glacier variety were used.

RESULTS.—No pectin methylesterase (PME) activity was detected in any of the culture filtrates of *C. fagacearum*. A 0.02 per cent preparation of commercial PME[3] used as a check showed considerable activity.

No polygalacturonase activity was found in any of the fungus culture filtrates; however, pectin depolymerase activity of the filtrates was high. When activity was expressed as relative increase in fluidity of 0.5 per cent pectin solutions (calculated by dividing the time

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Funds provided by the Pennsylvania Department of Agriculture in a special Oak Wilt Grant made this study possible.

[2] Associate Professor and Research Technician, respectively.

[3] Nutritional Biochemicals Corporation.

of flow, in seconds, of fungus culture filtrates into the time of flow of corresponding nonseeded culture medium), culture filtrates of Medium A showed an increase of 2.51; Medium A plus pectin, 2.75; Medium B, 2.91; and Medium B plus pectin, 3.46.

The quantity of pectin depolymerase produced was not affected by the kind of inoculum used. The presence of pectin in the medium stimulated enzyme production. In addition, more enzyme was produced in Medium B than in Medium A. Culture filtrates heated to 95°C for 10 minutes did not decrease the viscosity of the pectin test solution, nor did nonseeded culture media.

Culture filtrates of *C. fagacearum* caused a pronounced inward curvature of the split ends of the pea stems, an indication of the presence of growth-promoting substance or substances.

Ethylene was not produced by *C. fagacearum*. Comparable pea seedlings exposed to the emanations of *Penicillium digitatum* Sacc. as checks exhibited stunting, declination from the vertical, and swelling of the epicotyl tips—all characteristic indications of the presence of ethylene.

Discussion.—An important role has been proposed for pectic enzymes in inducing vascular plugging in Verticillium wilt (6), Fusarium wilt of tomato (3, 8, 10), and Dutch elm disease (1). It has been suggested that the hydrolysis of pectin substances in the middle lamella and primary cell walls by the fungal enzymes results in the production of large molecular fragments that aggregate in the xylary elements. This accumulation, accompanied by tylosis formation, occludes the water-conducting cells and induces wilting. The production of pectin enzymes may be involved in the formation of both gummous materials and tyloses, which have been observed in wilting northern pin oaks (2). Growth-promoting sugstances could be very important in a hypertrophic response, such as the formation of tyloses, by stimulating the protoplasm of the parenchyma cells. The production of pectin enzymes and of growth-promoting substance by *C. fagacearum* increases the list of wilt pathogens known to produce them; this further strengthens the concept of their hypothetical role in the induction of wilt symptoms in affected hosts.

The specific pectolytic enzyme produced *in vitro* by *C. fagacearum* is unknown, although herein it has been termed pectin depolymerase (7). Tests indicate that it is not polygalacturonase, a finding that is in agreement with results of studies by Beckman (1) on *C. ulmi*. Although *C. fagacearum* produced enzyme and growth-promoting substance in abundance *in vitro*, there is as yet no direct evidence that these are produced in wilting oaks.

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MIDLEAF NECROSIS — A VIRUS DISEASE OF SOUR CHERRY [1]

J. A. Milbrath

SUMMARY

A virus causing a disease not formerly recognized in stone fruits was isolated during an attempt to separate the sour cherry yellows virus from the ring spot virus by use of the Shiro-fugen variety of flowering cherry as a filter host. This virus caused large spreading necrotic areas on some leaves of Montmorency cherry. These areas were often limited to the midveins and adjacent tissue. The affected leaves either became chlorotic and fell from the trees or withered rapidly and remained attached to the branches for some time. Infected trees were markedly smaller in size and less vigorous than healthy ones, but fruit on them developed normally. An Olivet sour cherry and a Bing sweet cherry were found carrying the midleaf necrosis virus without symptoms. The disease also was observed in commercial sour cherry orchards in Oregon. The midleaf necrosis virus was recovered free from the peach ring spot and sour cherry yellows viruses; when the viruses were recombined, no evidence of relationship was obtained. Cherry midleaf necrosis is proposed for the common name of the disease and cherry midleaf necrosis virus as that for the causal virus.

Peach ring spot virus has been found to be consistently associated with the sour cherry yellows disease, but sour cherry trees (*Prunus cerasus* L.) frequently are infected with ring spot strains that do not cause yellows symptoms. Either 2 viruses are necessary for the expression of sour cherry yellows or this disease may be caused by atypical strains of peach ring spot virus. Cherry rusty mottle was separated from peach ring spot virus by the use of flowering cherry (*P. serrulata* Lindl.) as a filter host.[2] The rusty mottle virus moved rapidly into the Shiro-fugen and became systemic, whereas the ring spot virus moved slowly and killed the tissue as it advanced. Cultures of rusty mottle virus free of ring spot virus were obtained by selecting rusty-mottle-affected scion wood beyond the advancing necrosis caused by the ring spot virus. The same procedure was used in the experiments reported in this paper in an attempt to separate the virus or viruses that might be responsible for sour cherry yellows from that causing ring spot. Although the virus causing sour cherry yellows invaded the Bing (*Prunus avium* L. var. Bing) branch of the filter tree, no separation of the viruses causing ring spot and sour cherry yellows was accomplished. The sour cherry trees used as index hosts for the viruses in the Shiro-fugen filter tree failed to express yellows symptoms, and these trees did not become infected with peach ring spot. These Montmorency trees and progeny trees propagated from them, however, developed a midleaf necrosis symptom not formerly recognized as a stone fruit virus disease. The nature of this virus disease is reported in this paper.

SYMPTOMS OF MIDLEAF NECROSIS.—Symptoms of midleaf necrosis appeared on the oldest leaves at the base of the current season's growth. Symptoms became progressively more severe during the last 2 weeks in June, at which time 50 per cent or more of the leaves fell from the trees. After this heavy leaf fall, a few scattered leaves with midleaf necrosis were found during the rest of the growing season.

The first symptom to appear was a dark brown necrosis that usually originated in the midvein in the central portion of the leaf and then spread to the adjacent tissue (Fig. 1, B). Occasionally, the necrosis occurred near the base of the leaf and spread into the petiole. Such affected leaves usually withered rapidly before an abscission layer was developed and remained attached to the twig for some time. Occasionally, necrotic areas developed on some of the larger secondary veins (Fig. 1, A, C).

Later in the season, the leaves on affected trees often showed a chlorotic marbled pattern on a green background. This sometimes develops on leaves showing midleaf necrosis (Fig. 2, A) or on leaves without any of the typical necrotic areas (Fig. 2, B, C).

Four-year-old infected Montmorency trees, when compared to virus-free trees, were found to be retarded in growth and the date of blossoming was delayed several days. The fruit generally developed to a normal size and color.

No extensive host range studies have been made with the midleaf necrosis virus. Two each of Bing, Lambert, and Napoleon sweet cherry trees, 3 of Hale peach trees, and 5 of Shiro-fugen flowering cherry trees were inoculated with the midleaf necrosis virus but have failed to develop distinct symptoms. An Olivet sour cherry and a Bing sweet cherry were found carrying midleaf necrosis virus without symptoms.

ISOLATION AND RECOGNITION OF MIDLEAF NECROSIS VIRUS.—Midleaf necrosis virus was isolated and recognized as a distinct virus incidental to an attempt to separate sour cherry yellows virus from peach ring spot virus in the following experiment. A Shiro-fugen flowering cherry topworked with virus-free Bing on 4 separate branches was used as a filter tree. A Montmorency cherry tree, which was known to carry a necrotic strain of peach ring spot virus and which was showing leaf symptoms of sour cherry yellows, was

[1] Accepted for publication May 23, 1957.

Technical Paper No. 1042, Oregon Agricultural Experiment Station.

[2] Milbrath, J. A. 1951. Separation of rusty mottle of cherry from a ring spot rusty mottle complex. Science 113: 606-607.

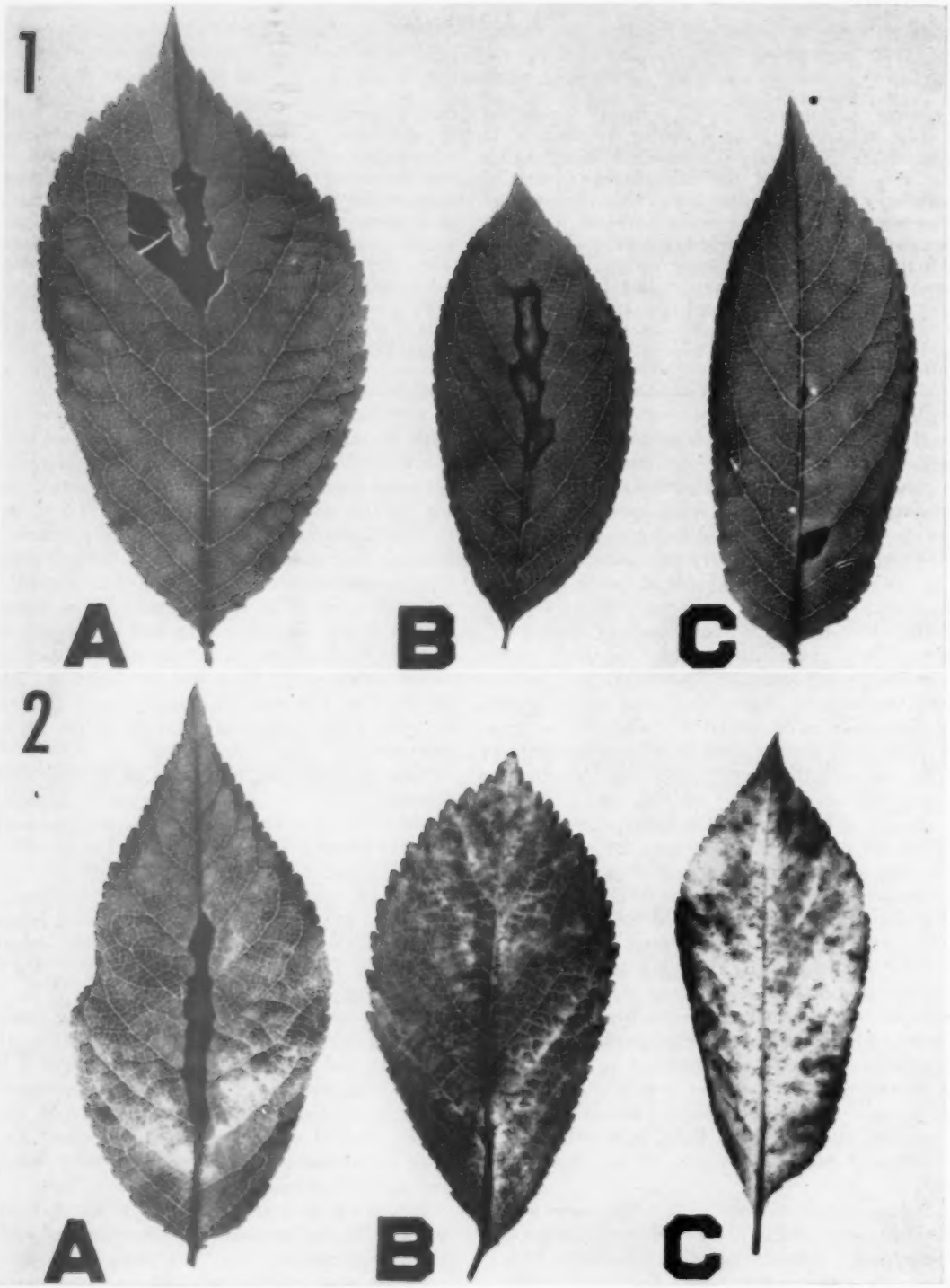

FIG. 1-2. Fig. 1. Montmorency cherry leaves showing the midleaf necrosis. A, C) Atypical types not located in the center portion of the leaf. B) Typical type of necrosis as it more commonly appears.—Fig. 2. Montmorency cherry leaves affected with the midleaf necrosis virus and showing the chlorotic patterns that develop during the latter half of the growing season. A) Midleaf necrosis and a secondary spreading chlorosis. B) A similar chlorotic pattern without necrosis. C) Advanced chlorosis just prior to leaf fall. (Photographs by H. H. Millsap).

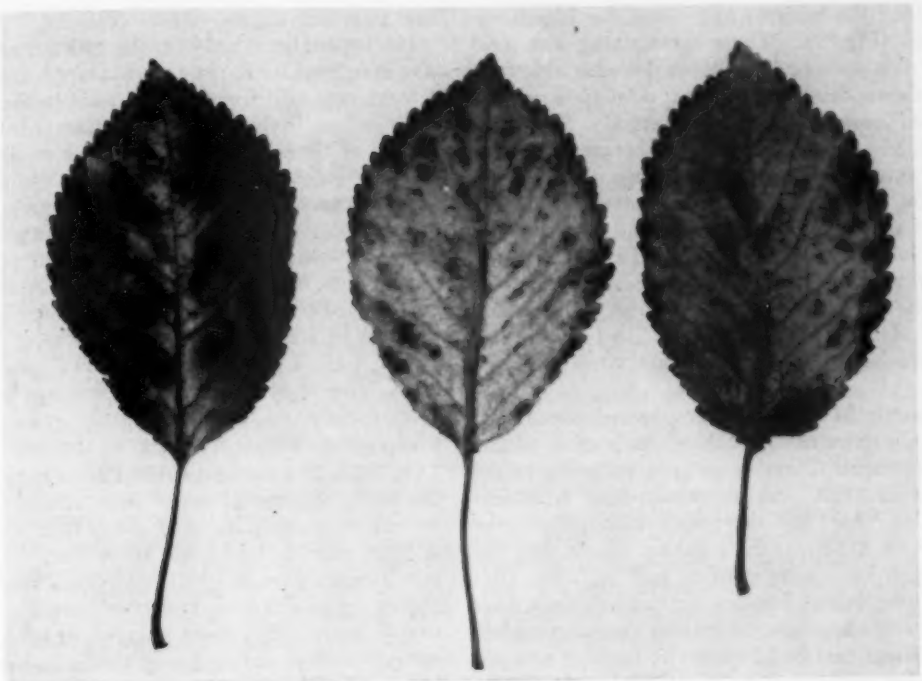

FIG. 3. Montmorency cherry leaves from branches inoculated with buds from Shiro-fugen filter tree April 23, 1949. These chlorotic leaf patterns suggested that a virus of the sour cherry yellows type had been isolated. (Photograph by H. H. Millsap).

used as the source of inoculum for these studies.

In September, 1948, 3 buds from the Montmorency tree were placed into one of the Bing branches on the filter tree. The Bing branch was inoculated to allow the virus components to become established in a non-necrotic host before entering the Shiro-fugen, because the ring spot virus would cause a necrotic reaction as soon as it infected the Shiro-fugen tissue. The ring spot virus has been shown[3] to move very slowly through Shiro-fugen, causing extensive necrosis and to result in death of the tree in most cases. It was assumed that other viruses present might move faster than the peach ring spot virus and could then be recovered from some of the Shiro-fugen branches not infected with the peach ring spot virus.

On March 23, 1949, the bark of the Shiro-fugen trees was slashed at and below the union with the inoculated Bing branches and examined for necrotic tissue, which would indicate the extent of infection by the ring spot virus. By this time, the necrosis had advanced throughout the Shiro-fugen arm bearing the inoculated branch and had started to invade some of the other arms at the crotch area. Scions were taken from the inoculated Bing branch and grafted onto a virus-free Montmorency. This tree subsequently became infected with peach ring spot virus and sour cherry yellows, proving that both viruses had invaded the Bing branch.

[3] Millbrath, J. A., and S. M. Zeller. 1945. Latent viruses in stone fruits. Science 101: 114-115.

Four other virus-free Montmorency trees were inoculated by grafting with scions from Shiro-fugen branches taken well beyond the advancing edge of the necrotic tissue. None of these trees became infected with peach ring spot virus, sour cherry yellows virus, or sour cherry midleaf necrosis virus, which indicated that none of these viruses had moved into the branches from which the scions had been taken. None of these trees have developed fruit symptoms of the recently reported[4] latent K and S virus now known to have been present in the Shiro-fugen filter tree before it was inoculated.

The Shiro-fugen tree was examined again on June 9, 1949, and 6 branches that still had good terminal growth beyond the advancing necrotic area were selected. Single virus-free Montmorency trees were inoculated by means of 5 buds from each of the branches; all were placed on 1 arm.

The first evidence that a virus had been transmitted to the Montmorency was noted on August 18, 1949. Four of the 6 Montmorency trees inoculated April 23, 1949, showed yellowing of some of the oldest leaves near the points of inoculation (Fig. 3). A purple flecking appeared first on the upper surface of the leaf, and this was followed by epidermal bronzing on the under surface of the leaf. This discoloration was

[4] Reeves, E. L., P. W. Chency, and J. A. Milbrath. 1955. Normal-appearing Kwanzan and Shiro-fungen cherries found to carry a virus of little cherry type. Plant Disease Reptr. 39: 725-726.

strongest along the midvein and along the larger secondary veins (Fig. 3). Where the flecking was most prominent, the surrounding tissue became chlorotic. The chlorosis was faint at first, but it developed rapidly. Such affected leaves were shed prematurely. Just prior to leaf fall, most of the leaf blade became chlorotic except for green borders surrounding the necrotic flecking. The general leaf pattern was quite distinct from that of sour cherry yellows, but the chlorosis and the falling of older leaves were suggestive that the sour cherry yellows virus might have been involved. No symptoms indicative of ring spot were seen on any of the 6 inoculated Montmorency trees, and later indexing on Shiro-fugen proved that this virus was not present.

Approximately 35 trees were propagated on mazzard roots from the terminal growth of each of 4 of the Montmorency trees. These trees grew normally in the nursery row in 1950, and no outstanding symptoms were recorded. In 1951 they were moved from the nursery row to a permanent planting where they became established and were growing well by June. That these trees were infected with a new and distinct virus became apparent towards the end of the month when more than 50 per cent had leaves with midleaf necrosis symptoms. On some trees, only a few leaves showed midleaf necrosis, whereas on others many of the leaves were affected. This difference in degree of severity has continued to show during the past 6 years, indicating that different strains might have been isolated in the individual Montmorency trees. When the 4 bud-source Montmorency trees were reexamined, midleaf necrosis was very apparent on many leaves.

The progeny of the 4 trees were reinoculated with 6 different strains of the peach ring spot virus, 3 of which also caused sour cherry yellows symptoms when normal Montmorency trees were inoculated. These 3 strains also caused yellows symptoms when introduced into the trees with midleaf necrosis. This combination of viruses greatly reduced the growth of the trees. Some of these inoculated trees were only 3–4 ft. tall after 4 years of growth, whereas virus-free Montmorency were 8–9 ft. tall. The trees that had midleaf necrosis when inoculated with the 3 strains of ring spot virus that did not induce yellows when inoculated into the normal trees also did not show yellows. Therefore, midleaf necrosis virus was not a part of the ring spot plus sour cherry yellows complex.

On September 15, 1949, the Shiro-fugen filter tree was examined for the last time. Only 1 arm, which had 3 forks, was still free from necrosis in the bark tissue. Buds taken from the basal portion of the terminal growth of these 3 forks indexed free of ring spot virus on Shiro-fugen. Other buds from the terminal portions were placed on 3 virus-free Montmorency trees. None of the Montmorency trees developed ring spot, an indication that the ring spot virus was not present in the Shiro-fugen branches when the buds were cut. Later indexing of the inoculated Montmorency trees showed them to be free of the ring spot virus, a further indication that ring spot virus was not present in these trees. The Montmorency trees developed typical midleaf necrosis and therefore must be presumed infected with a virus distinct from that of ring spot.

By 1951, 2 of the 3 inoculated Montmorency trees developed symptoms of some virus complex. The trees appeared sparsely foliated, numerous leaves showed midleaf necrosis, and some leaves were chlorotic; however, there were no leaf symptoms typical of sour cherry yellows. These trees have been observed for the past 6 years. They were delayed in blossoming, fruit spurs failed to develop, and the branches assumed a weeping habit similar to trees with sour cherry yellows. The fruit ripened unevenly and often was lighter in color and smaller than that on adjacent virus-free Montmorency trees. Progeny from these 2 trees have been indexed on Lambert sweet cherry and were found to carry the K and S latent little cherry virus; therefore, the Shiro-fugen filter tree apparently was infected with the K and S virus. The reason why only these 2 Montmorency trees of this set and none of the 6 trees in the previous set, which had been inoculated directly from the Shiro-fugen filter tree, became infected is not known. The possible contamination of these trees with the K and S little cherry virus had not been suspected until this work was nearly completed. The 2 Montmorency trees inoculated on September 15, 1949, apparently became infected with a combination of midleaf necrosis and K and S little cherry viruses, but they were not infected with peach ring spot virus and did not develop typical sour yellows symptoms.

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CEPHALOSPORIUM STRIPE DISEASE OF WHEAT [1]

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SUMMARY

Cephalosporium stripe, a disease that has been locally destructive on winter wheat and barley in Japan, has been found in Washington on winter wheat, *Dactylis glomerata*, *Bromus marginatus*, *Elymus glaucus*, *Secale cereale*, and *Agropyron repens*. Inoculations in the greenhouse resulted in infection of members of the following genera: *Agropyron*, *Arrhenatherum*, *Avena*, *Beckmannia*, *Bromus*, *Dactylis*, *Elymus*, *Hordeum*, *Lolium*, *Oryzopsis*, *Phleum*, *Poa*, *Polypogon*, *Secale*, *Sitanion*, and *Triticum*. The disease is most prevalent where soils are moist, is widespread geographically, and is not closely associated with a given soil type. Observations to date show that stripe disease is of economic importance.

Washington isolates of the pathogen *Cephalosporium gramineum* had the same cardinal temperatures as did those in Japan (5°, 20°, and 30°C). The spores of isolates from the 2 regions were similar in size and shape. Most of the Washington isolates were similar in cultural characteristics, but the finding of a few yellow-orange individuals indicates that in Washington the species varies more than it does in Japan. The fungus is slow growing in culture; sporulation takes place within and upon the medium.

Infection can result from invasion through underground structures when wheat is grown outdoors in naturally infested soil. The fungus invades the entire aboveground portion of the plant through the vascular system. Cultures are most easily obtained from leaf and culm tissues taken well above the soil. Few caryopses were found to be invaded, and infected seed probably serves only to introduce the fungus into new areas. Infested straw remains a source of inoculum under dry conditions for at least a year.

Spring wheat past the seedling stage and well-vernalized winter wheat appeared to be more susceptible in hypodermic-inoculation trials than did young wheat plants. In field-grown wheats, symptoms varied from prominent leaf stripes and a few white, blighted heads to inconspicuous leaf stripes and many white, empty heads in other varieties. Preliminary results offer hope of some control through resistance.

Cephalosporium stripe disease of wheat was first observed in Washington (3) in 1955. Field observations and inoculation trials have both demonstrated the severity of the disease. The importance of wheat to the agriculture of this state prompted the study of this disease and of the pathogen, *Cephalosporium gramineum* Nisikado & Ikata, even though they had received thorough study in Japan. Since our climate, soils, and cultural methods are different from those in Japan, much of the basic Japanese work was repeated with Washington cultures of the fungus and with wheats adapted to our region to determine how closely Japanese findings would be applicable here.

Symptoms and losses in Washington varieties and in our environments should be determined and adapted resistant varieties found or developed. The potential of the pathogen, its variation, and its distribution within the state should be known. Exploratory experiments designed to answer these questions are presented, along with techniques for investigating the disease.

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REVIEW OF LITERATURE.—Stripe disease of wheat was first noticed by farmers near Kurasiki, Japan (15). Exact knowledge of the disease began with the observation by Nisikado (15) in 1932 that the xylem vessels of striped leaves and culms contained a vascular parasite, which was described as a new species, *Cephalosporium gramineum*. Stripe disease became important following the expansion of wheat culture under an intensive 5-year self-sufficiency program, wherein wheat was grown every year on the same fields in some areas (15). The disease became severe in Okayama and Kagawa Prefectures, adjacent to the Inland Sea; this led to intensive study, a comprehensive report of which appeared in 1934 (15). In this report, Nisikado et al described the disease in detail, discussed the taxonomic position of the fungus, and presented their observations on factors influencing disease development. Infested stubble, straw, and soil were listed as the major sources of inoculum. Seed was reported as serving a minor role as a source of inoculum in wheat and barley. Early fall seedlings of wheat were reported as more severely affected than late seedlings. Crop rotation, destruction of stubble and straw, and selection and breeding of resistant varieties were given as promising control measures. Apparently, many grasses were found to be hosts, but only wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), *Avena fatua* L. (15), and *Alopecuris agrestis* L. (13) were specificially mentioned. The choice of the specific epithet *gramineum* implies a wide host range. Nisikado and Higuti (13) postulated that the temperature requirements (optimum, 20°C) of this organism indicated origin in a

temperate region.

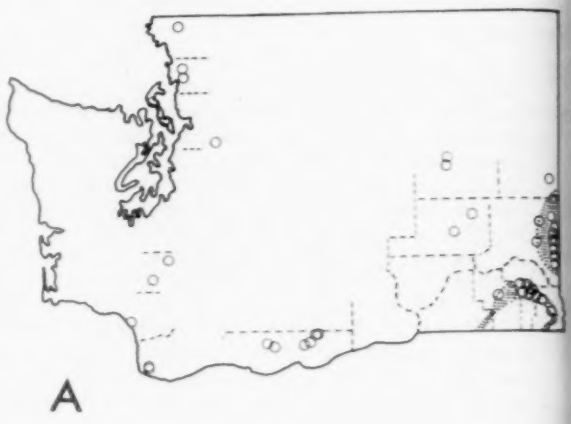
Ikata and Kawai in 1937 (12) found that *C. gramineum* invades the plant through roots and that it produces toxic materials in culture that inhibit seedling growth. Nisikado et al (14) reported that the fungus withstood adverse temperatures better in the dry state than when moist. Ikata et al (11) found that the pathogen (in infested barley straw and grain) passed unharmed through the alimentary canal of cattle and poultry.

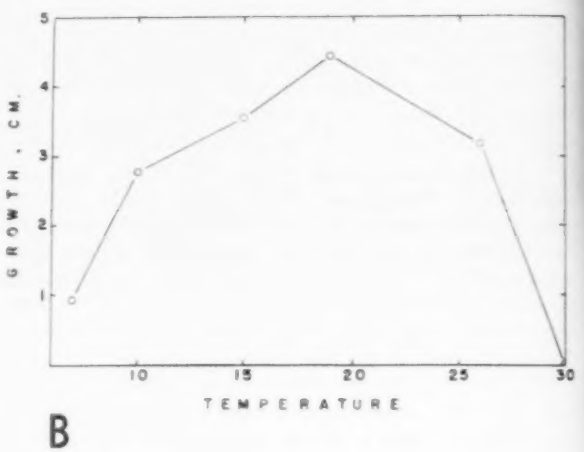
A more recent summary of observations in Japan, published in 1946 (19), showed that the area of infestation increased from 3000 hectares in 1934 to 5000 in 1941 and that these fields produced little or no grain. Practical control—by use of clean seed, seed treatment, rogueing, avoiding composts of infested straw, crop rotation, and late seeding—had by 1944 reduced the area of appreciable loss to 2000 hectares, but by then stripe had appeared in new localities in Honshu and Shikoku. The Japanese apparently have succeeded in controlling this disease.

DISTRIBUTION IN WASHINGTON.—The Cephalosporium stripe disease was discovered in Washington late in the season of 1955 at 4 widely scattered points on the Elmar variety of white winter club wheat. A more extensive search in 1956 showed that the disease was widespread on Elmar, Brevor, and Golden. Stripe was found most often in areas receiving drainage water (4).

In the subhumid, highly productive areas of southeast Washington, the incidence of stripe on wheat was high, particularly in moist localities along the eastern border and near the timbered outcrops of the Blue Mountains. These areas are rather high, have 20–30 in. of rainfall, and have relatively low mean annual temperatures (Fig. 1.A). Annual recropping to wheat and wheat following summer fallow are common practices in this area.

In Klickitat County, to the west of the important Horse Heaven dryland wheat area, stripe was found in the most moist part of the grass-to-timber transition area, where annual rainfall ranges from 15–30 in. In contrast to the eastern Washington grain area, the soils in this area are less fertile, a more general type of





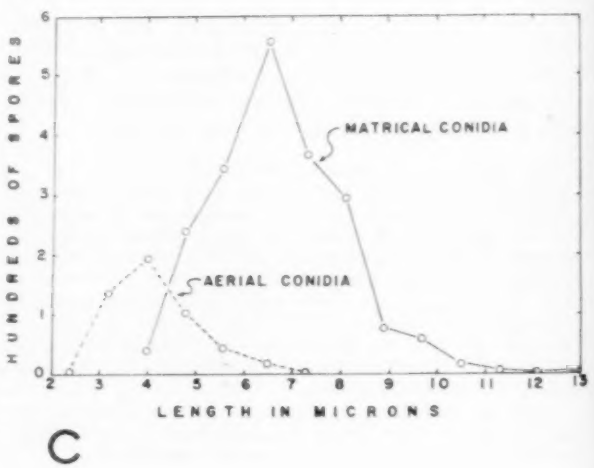


FIG. 1. A) Known distribution of Cephalosporium stripe disease of wheat in Washington. The circles indicate points where the disease was found on wheat or grass (1955 and 1956 surveys). The disease was found most frequently in wheat lands with an annual precipitation of 20–30 in. (shown by shading). The county lines shown give some measure of the area surveyed. B) Diameter of mycelia of *Cephalosporium gramineum* incubated on potato-dextrose agar for 3–4 weeks in Petri dishes at different temperatures (°C). The means of 4 isolates (Bickleton, Dayton, Garfield, Wilbur), at least 6 replicates of each, are given. The individual isolates showed no significant variations in temperature requirements. C) Frequency of occurrence of various conidia lengths in *C. gramineum*, when conidia were collected from the surface of the medium (matrical conidia) and from hyphal "bristles" (aerial conidia). Measurements were made of 2000 matrical and 500 aerial conidia.

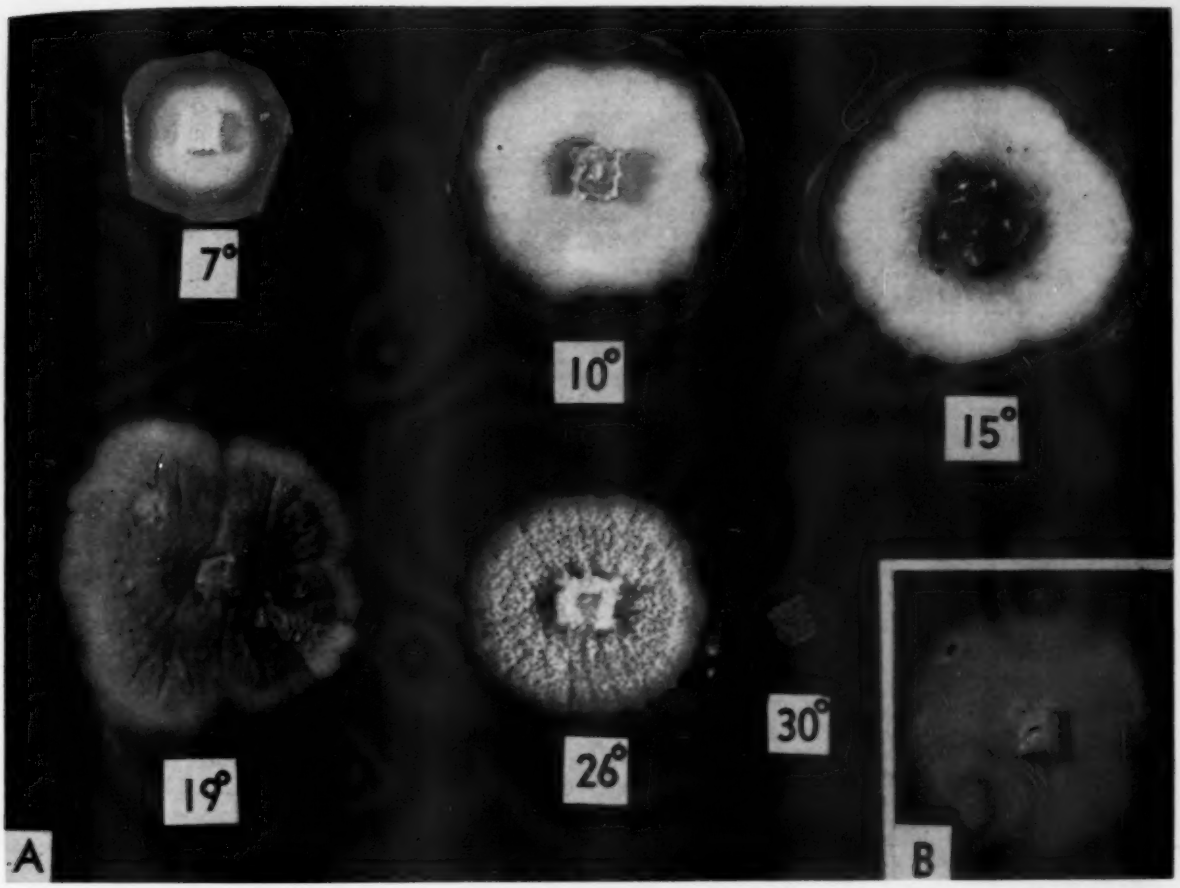


FIG. 2. A) The influence of temperature upon cultural characteristics of *Cephalosporium gramineum* (Garfield isolate) grown 4 weeks on potato-dextrose agar. Color and aerial hyphae develop above 15°C. but only minute pyramidal mounds were formed at 30°. B) Bickleton isolate incubated at 15°C. Note the abundant slimy sporulation.

farming is practiced, and considerable land is planted to alfalfa and grass hays.

West of the Cascade Mountains, *C. gramineum* was found in an occasional striped plant of *Dactylis glomerata* L. and of winter wheat in Clark and Skagit counties. Wheat was scarce in this area, and all collection points are quite humid, with the exception of 1 site near Coupeville, Island County, where the annual rainfall is only 15–20 in.

In the true dryland region, the semiarid plains area of central Washington, stripe has been seen in only 2 locations. In 1955, striped Elmar was found in a low spot in a Lincoln County field south of Wilbur and in 1956 in experimental plots at Lind, in Adams County. At this latter site, the wheat was space-planted on summer fallow in rows 2 ft. apart and 6 in. in the row, thus giving a higher than normal moisture supply. An isolate of *C. gramineum* was obtained from the base of a prematurely blighted plant of Rio winter wheat east of Ritzville. The veins of the culm were not discolored, and the leaves were blighted to a degree that prevented detection of stripe.

These preliminary observations indicate that the pathogen is widespread in the state, particularly in relatively cool and humid areas, and that no close relationship to a particular soil type exists.

CULTURAL STUDIES ON CEPHALOSPORIUM GRAMINEUM. —*Temperature relations.*—Four winter wheat isolates were incubated on potato-dextrose agar at temperatures ranging from 7° to 35°C. Gum-rubber seals were placed around the Petri dishes to minimize water loss. The inoculum consisted of small agar blocks containing vigorous mycelia. Each block was placed at the center of a dish. After an incubation of 3–4 weeks, the mycelia were measured and the amount of sporulation observed.

Mycelial development occurred from 7° to 27°, with the optimum about 19° (Fig. 1, B). At cool temperatures (7°-15°), the mycelial colony was white to creamy, had a slimy surface, and had almost no aerial hyphae (Fig. 2). Sporulation at 7° was so abundant and aqueous that the slimy spore mass occasionally flowed when the dishes were tipped on edge. Sporulation diminished with rising temperatures, whereas formation of aerial hyphae increased. A gray-tan color developed at 20° and above. Aggregates of entwined

TABLE 1.—*Length of spores of Cephalosporium gramineum produced on potato-dextrose agar*

	Length of conidia (in μ)[a] when fungus was grown at indicated temperature (°C)								
	Conidia on or within matrix						Aerial conidia on hyphal "bristles"		
Isolate	7°	10°	15°	19°	25°–27°	Average	15°	19°	Average
Bickleton	7.09	6.84	6.79	7.33	7.21	7.05	4.10	3.93	4.01
Dayton	6.71	6.34	6.15	6.14	5.44	6.16			
Garfield	7.96	6.73	7.17	7.33	7.91	7.42	4.29	4.26	4.27
Wilbur	6.02	6.01	6.38	6.35	6.36	6.22	4.37		4.37
Average	6.95	6.48	6.62	6.79	6.73	6.71			

[a] Each figure represents the mean of 100 spores.

aerial hyphae formed "bristles," or "whiskers," visible to the unaided eye. Sporulation occurred on lateral conidiophores arising as branches from these bristles. The mycelia frequently developed a furrowed surface, either radiate-straight or spiral. At 30°, no measurable mycelial extension occurred, but a crusty tan layer with minute projections and some sporulation developed on the inoculum pieces (Fig. 2).

Growth resumed when plates held at 35° during the incubation period were transferred to room temperature, an indication that desiccation did not inhibit growth and that 3–4 weeks at 35° was not lethal.

Conidia.—Previous measurements of a small number of conidia (3) showed their lengths and proportions, length to width, to be similar to those of conidia of Japanese isolates. A further check on conidial size, based on spore length, was made with spores from the preceding temperature study. Aerially formed conidia were obtained from bristles removed by means of forceps. Matrical conidia, from the surface of the medium or within the matrix, averaged considerably larger than did aerial conidia borne on the drier bristle site (Table 1, Fig. 1, C). This type of variation in conidial size had been seen in *Cephalosporium* previously by Buchanan (5). The conidia borne on or within the matrix are those ordinarily measured, and the measurements obtained agree closely with those given for *C. gramineum* (13, 15). Matrical conidia stained with the Giemsa stain were uninucleate. Anastomosis of conidia, like that observed by Butler and Khan (6) in *C. sacchari* Butl., was seen in *C. gramineum*. Conidia were abundant both within the medium and upon it.

Conidiophores.—The conidiophores of *C. gramineum* varied from short projections about as long as a spore to very long. Mucilaginous spore heads formed on aerial conidiophores under moist conditions.

Color.—Thirty-three isolates have been studied on potato-dextrose agar at 20°C. At 60 days of age, 31 of these cultures varied from white to dark gray, 1 had lavender tints, and 1 was yellow-orange. One of the gray isolates produced a yellow sector. The 2 isolates with bright colors and the the yellow sector produced typical stripes on wheat, and there is no reason for considering them anything other than *C. gramineum*. Dull colors definitely predominate, but this cannot be taken as a specific character.

HOST RANGE.—In Washington fields, stripe has been found not only on wheat and rye but also on the grasses *Agropyron repens* (L.) Beauv., *Bromus marginatus* Nees., *Dactylis glomerata* L., and *Elymus glaucus* Buckl. All isolates from grass have proved pathogenic to wheat, inducing symptoms similar to those induced by isolates from wheat. One wheat isolate was injected into Tendermost sweet corn (12–18 in. tall) in several points along the stem. No evidence of disease was seen.

A sizeable host range was indicated by a preliminary greenhouse trial in which spore suspensions of a single isolate from wheat were injected into culms. Leaf stripes, brown vascular bundles, and recovery of the pathogen at a distance from the point of injection were the criteria of susceptibility. The following grasses were found to be suscepts: *Agropyron cristatum* (L.) Beauv., *A. inerme* (Scribn. & Smith) Rydb., *A. repens* (L.) Beauv., *A. trichophorum* (Link) Richt., *Arrhenatherum elatius* (L.) Presl., *Avena fatua* L., *A. sativa* L., *Beckmannia sp.*, *Bromus catharticus* Vahl., *B. marginatus* Nees, *B. mollis* L., *B. rigidus* Roth., *B. tectorum* L.,*Dactylis glomerata* L., *Elymus condensatus* Presl., *E. glaucus* Buckl., *E. junceus* Fisch., *Hordeum vulgare* L., *Lolium perenne* L., *Oryzopsis hymenoides* (Roem. & Schult.) Ricker, *Phleum pratense* L., *Poa ampla* Merr., *P. canbyi* (Scribn.) Piper, *P. compressa* L., *Polypogen monspeliensis* (L). Desf., *Secale cereale* L., *Sitanion hystrix* (Nutt.) J. G. Smith, *S. jubatum* J. G. Smith, and *Triticum aestivum* L.

Stripes ranged from red (as on Monarch oats) through brown, tan, and yellow to chlorotic stripes (as on wheat). Blighted inflorescences occurred in *Bromus rigidus*, barley, *Sitanion* spp., wheat, *Polypogon monspeliensis*, and rarely in oats. Oats were quite resistant, barley was intermediate, and wheats were the most susceptible and the best diagnostic hosts. The stripes on barley leaves (15) and on oats were less distinct than those on wheat (Fig. 3).

ORGANS AND TISSUES INVADED BY CEPHALOSPORIUM GRAMINEUM.—The distribution of the fungus within the host was determined by isolation of the pathogen from selected structures. The slow-growing nature of the fungus rendered attempts to obtain it from crowns and roots of naturally infected plants unsuccessful, but a complete invasion of aboveground parts was demonstrated. Routine isolations from field collections con-

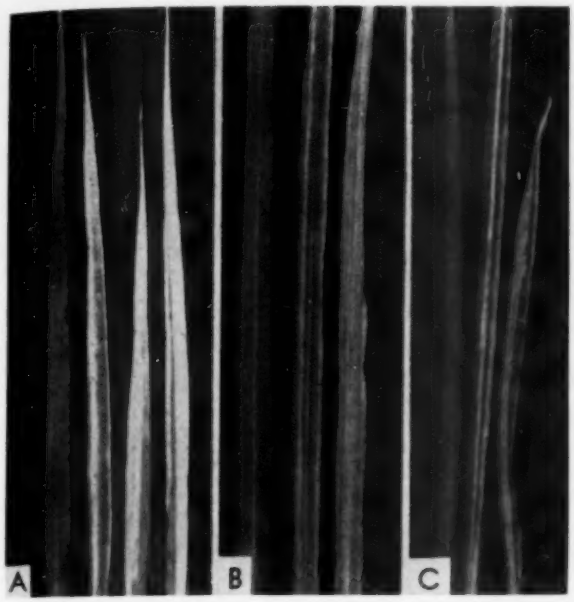


FIG. 3. Cephalosporium stripe disease on blades of A) Rojo barley, B) Shasta oats, and C) Federation wheat. Note that the stripes are quite diffuse in barley, fairly sharp in oats, and most distinct in wheat. In each set, the leaf on the left is from a healthy plant.

sistently gave cultures from striped leaves and from culms of striped plants. In more detailed greenhouse studies, inflorescences were dissected and plated; the fungus was found in all portions of the spike (Table 2).

Failure to recover *C. gramineum* from seeds of naturally diseased Elmar in the field was reported in 1955 (3). Its occurrence in seed of inoculated plants in the greenhouse led to a recheck of field-grown seed of the 1956 season. Fully matured seed of Kharkof, Elgin, Elmar, Brevor, and Golden varieties (587 seeds in all) from stems hypodermically inoculated near the ground were surface treated for 1 minute in a mixture of equal parts of 95 per cent ethyl alcohol and Clorox (5.25% sodium hypochlorite), plated on corn-meal agar, and incubated 12 days at 10°C. Five of the 587 seeds yielded cultures of *C. gramineum;* thus only 5 of 714 seeds yielded the fungus in 1955 and 1956. Seed from diseased plants was sown in the fall of 1956; in the spring of 1957 a few striped plants developed, showing that such seed can serve to introduce the pathogen. Greenhouse-grown diseased plants have a fairly high proportion of infected caryopses (Table 2). The results to date suggest that the pathogen may be introduced to new areas by means of seed but that the percentage of natural seed infection is too low to produce an epidemic.

Free-hand sections of upper culm tissues confirmed Japanese reports that *C. gramineum* is primarily a xylem invader and that sporulation occurs within large xylem vessels (15). In advanced stages of disease development, the fungus was observed in the ground meristem and even in the culm cavity, where it formed a smoky-gray mycelium with some sporulation. Occasionally, the xylem vessels were so filled with mycelium as to appear a real hindrance to movement of fluids. The brown discoloration of vascular elements occurred where little mycelium was present, indicating an upward movement of harmful metabolites from heavily parasitized areas.

TABLE 2.—*Isolation of Cephalosporium gramineum from upper shoot parts of greenhouse-grown plants of wheat, oats, and barley inoculated near the base*

Plant structure	No. of isolations that were positive (+) or negative (−)					
	Wheat		Oats		Barley	
	+	−	+	−	+	−
Awns					3	7
Caryopsis or "seed"*	16	111	3	72	39	87
Paleas	1	14			0	7
Lemmas	7	9			2	4
Glumes	7	9			5	3
Rachillas	3	9			4	7
Rachis segments	32	2			11	0
Top, last culm internode	16	0			4	0
Middle, last culm internode	15	1			4	0
Bottom, last culm internode	16	2			4	0

* In oats and barley, the lemmas and paleas adhered to the "seeds" and are included.

SURVIVAL OF THE FUNGUS IN STRAW.—The general invasion of the plant suggests that straw and stubble may perpetuate the pathogen. The Japanese reported survival of the fungus in steamed infested rice straw for 34 months at 0°–10°C and for shorter times at higher temperatures (14). Experiments were conducted to explore the ability of *C. gramineum* to survive in straw long enough to bridge the period between harvest and the succeeding fall sowing or the next year.

Straws of hypodermically inoculated greenhouse-grown wheat that had been stored in an office in a dry atmosphere were cut into small segments after 2, 4, 8, 12, and 16 weeks of such storage. The segments were dipped in sterile water to permit some hydration, then placed for 1 minute in the mixture of alcohol and sodium hypochlorite previously described, and finally transferred directly to 2 per cent water agar in Petri dishes. Following incubation at room temperature, the fungus was obtained from some pieces at all intervals tested (26 of 31 at 2 weeks, 31 of 33 at 4 weeks, 20 of 30 at 8 weeks, 19 of 42 at 12 weeks, and 30 of 46 at 16 weeks).

Straws of naturally diseased field-grown Elmar stored for 1 year in an office yielded the fungus (25 of 37 pieces plated). A sample of naturally infested straw of field-grown Elmar, cut in July and stored in a greenhouse headhouse until September, was scattered upon the soil surface and covered sufficiently with soil to hold it from blowing away. Seventy per cent of the Elmar plants in the strawed area became striped, whereas only 10 per cent of those in an adjacent unstrawed area were diseased. The results of this limited test, coupled with Japanese experience and the above isolation experience, are strong evidence

that infested plant parts perpetuate this pathogen.

INVASION THROUGH UNDERGROUND PORTIONS.—The role of infested soil in perpetuating the stripe pathogen (15) strongly indicates that infection can result from invasion through subterranean structures. Ikata and Kawai (12) saw the pathogen in vessels of large roots adjacent to the crown, and they reported infection following root inoculation. In further tests of the role of infested soil in stripe disease, both naturally and artificially infested soils were employed.

Infested soil in the field.—Naturally infected plants of Elmar wheat showing stripe were tagged in a field near Garfield. Just before harvest, these plants were dug from the very dry soil. The soil immediately around the base of individual diseased plants was saved, along with a 2-in. stubble. This material was pulverized, bulked, mixed, and stored dry until fall. In September (about the time of the fall rains) this soil was placed in 2 flats containing 2 × 2-in. wooden plant bands and then moistened. One flat was steamed, the other was not. Elmar wheat was sown in both flats on September 15, and the seedlings were transplanted to the field at Pullman on October 7. The plant bands were left in place to hold the soil about the growing wheat. On June 14, 1956, all 53 plants originating from the steamed flat were healthy, whereas 31 of 41 originating from the unsteamed flat were diseased.

Artificial infestation of soil in the field.—The pathogen was grown 4–5 days at room temperature in 1 liter of a potato decoction with dextrose in 2-liter flasks that were shaken at least once each day. This inoculum was injected into the soil 3–4 in. below the soil surface, under wheat in the jointing stage, at Pullman and Pomeroy, Washington. Soil moisture at the time was about optimum for wheat. Thirty ft. of wheat row was injected at each location. Since the injector had a gently tapered tip that produced a minimum of root injury, severe root injury was effected in half of each row by means of a common table knife inserted about 4 in. deep close to the bases of the plants. Results were quite variable: infection varied from a trace to 50 per cent in different portions of the row. Wounding with the knife increased infection: at Pullman, 55 culms in the unwounded portion of the row were striped, and 107 were striped in the wounded portion. Results at Pomeroy were similar.

Artificial infestation of soil in the greenhouse.—Incorporation of Petri-dish cultures of *C. gramineum* into soil in the greenhouse resulted in very little disease in wheat plants grown in such soil. These trials included cultures above, at, and below seed level, pasteurized and unpasteurized soil, and soil temperatures from 18° to 30°C. Only 12 of at least 300 plants became diseased. The fungus was present in these soils, even at the end of the experiments. No satisfactory explanation can be given for the success of field inoculations and this failure of greenhouse trials. Perhaps some critical environmental factor was lacking in the greenhouse. If root injuries are very important in inoculation, the lack of freezing and thawing, drought cracks, injurious cultural practices, or insect activity in the greenhouse might account for the results.

TABLE 3.—*Empty white heads observed*[a] *on winter wheat inoculated by germination of the seeds on Petri-dish cultures of Cephalosporium gramineum and grown at the Dry Land Experiment Station, Lind, Washington, 1955-56*

Variety	Treatment	No. of plants	No. of white heads	No. of white heads per plant
Kharkof	Inoculated	73	261	3.57
	Noninoculated	25	6	0.24
Rio	Inoculated	87	257	2.95
	Noninoculated	49	10	0.20
Comanche	Inoculated	5	6	1.20
	Noninoculated	18	4	0.22
Brevor	Inoculated	15	0	0.00
	Noninoculated	41	0	0.00
Elmar	Inoculated	24	8	0.33
	Noninoculated	9	0	0.00
Elgin	Inoculated	10	2	0.20
	Noninoculated	11	0	0.00

[a] Data taken June 13, 1956.

Inoculation by germination of seeds on mycelia in Petri-dish cultures.—Eighteen to 63 per cent infection resulted when Nisikado and Higuti (13) germinated wheat on cultures held at 5°, 10°, or 15°C and then transplanted the seedlings when the plumule was 1–3 mm long. In a greenhouse trial in Washington, this technique gave only a trace of infection (3). A similar field trial, in which 4 isolates and several varieties germinated at 10°–20°C were used, resulted in almost 100 per cent infection. In this trial, the seedlings were removed from the Petri dishes when the plumules were at least 3 mm in length, transplanted to plant bands, and subsequently transplanted to the field at Lind and Pullman, Washington, in the fall of 1955. At Lind, a dry location, blighting, with resultant empty heads, prevailed in some varieties (Table 3). Severe winter killing gave erratic plant numbers. At Pullman, a relatively humid site, stripe was pronounced, but blighting of heads was less prevalent.

GROWTH STAGE OF HOST AS A FACTOR INFLUENCING INFECTION FROM HYPODERMIC INOCULATION.—Hypodermic inoculations with spore suspensions established the pathogenicity of *C. gramineum* in spring wheat (2, 3). These initial successes led to further trials with winter wheat, since winter wheat is more important than spring wheat in Washington.

Seed of 10 varieties of winter wheat was germinated, vernalized for 4 weeks, and then sown September 9 in 6-in. standard clay pots in unsterilized soil. Six plants of each variety were inoculated hypodermically about 1 in. above the soil line on September 27 and an additional 6 plants on October 26. Twelve noninoculated plants of each variety served as checks. The wheat was grown at 65°F and no supplemental light was given. Water and nutrients were ample.

Since the wheat was poorly vernalized, all but 2 varieties, Atlas 66 and Norin 10, remained vegetative

TABLE 4.—*Development of stripe disease in Baart spring wheat and in Elmar and Rio winter wheats following hypodermic inoculation at different stages of growth*

Variety and treatment of seed	Date planted	Stage of development when inoculated (Feb. 10)	Disease development: Relative degree of leaf striping (Feb. 25)	Disease development: Plant development	Disease development: No. striped shoots or culms[a]
				(March 9)	(March 9)
Baart	Nov. 25	Flag leaf just showing	+	Anthesis completed	32
	Dec. 17	Jointing, 6 leaves	++	Anthesis beginning	42
	Dec. 30	Jointing, 5-6 leaves	+	Plants 16 in. tall	34
	Jan. 1	4 leaves	—	Plants 12 in. tall	20
	Jan. 20	3 leaves	—	Plants 8 in. tall	5
				(Feb. 25)	(March 8)
Elmar					
Vernalized	Dec. 16	Tillering, 5 leaves	+	Jointing	36
Nonvernalized	Dec. 16	Tillering, 5 leaves	+	Rosette	0
				(Feb. 25)	(April 16)
Rio					
Vernalized	Dec. 16	Rosette, many tillers	+	Jointing	78
Nonvernalized	Dec. 16	Rosette, many tillers	—	Rosette	33

[a] Of an approximate total of 96 culms per treatment.

an abnormally long time. Several plants of these 2 wheats, probably not true winter wheats, developed stripes, and several heads blighted. There was almost no infection in the other varieties (Elmar, Rio, Rex, Brevor, Comanche, Wasatch, Triplet, and Kharkof). The second inoculation date was slightly superior to the first. These results suggested that a long vegetative phase was not conducive to good infection and disease development; perhaps the partially vernalized winter wheats were not susceptible. To check this hypothesis, a second series of tests was run.

Baart, a spring wheat, was sown on November 25, December 17 and 30, and January 9 and 20. Elmar and Rio winter wheats, vernalized and nonvernalized, were sown December 16. Sixteen plants of each winter wheat, vernalized and nonvernalized, were inoculated February 10. Eight noninoculated plants of each set served as checks. Eight Baart plants of each seeding were likewise inoculated the same day with portions of the same spore suspension, and an equal number noninoculated plants served as checks. At that time, the vernalized and nonvernalized winter wheats were both in the rosette stage; Baart ranged from the 3-leaf stage to the flag-leaf stage.

Stripes were visible in the vernalized winter wheat and in the more advanced Baart 15 days after inoculation. From this time on, stripe was more abundant in the vernalized winter wheat, and in the Baart inoculated past the seedling stages, than it was in the nonvernalized winter wheat and in Baart inoculated in the seedling stage (Table 4).

This indication of resistance in the plumule of seedling spring wheat and in nonvernalized winter wheat in the rosette stage seems at odds with the success obtained by germinating winter wheat on mycelia and transplanting it to the field. It is probable that the fungus is not particularly active until the host passes a certain stage of development.

REACTION OF WHEAT VARIETIES TO HYPODERMIC INOCULATION.—Vigorous and well-fertilized winter wheats growing at Pullman and near Pomeroy, Washington, were inoculated on 2 dates: in an early jointing stage and in a later jointing stage. At Pullman, the stems of plants in 6 ft. of row and in 2 ft. of row of each variety were inoculated at the first and second dates, respectively; at Pomeroy, inoculations were made in 4 ft. of row on each date. Rich spore suspensions were injected into the stems about 2 in. above the ground; the Garfield isolate was used at Pullman, the Dayton isolate at Pomeroy. The wheat was about 2 ft. tall on the second date, and the stems of some varieties were hardening. Infection rates were high following inoculation at both dates. At maturity, the grain was threshed on a rub-board and breath-winnowed to save the light chaffy kernels.

Leaf stripes were evident before the plants headed, but the stripes became more abundant and striking with further development of the host. Unlike the common root and crown rots, symptoms varied considerably with the variety of wheat, and yields were affected differentially. Percentage yield reductions, by varieties and with the data for the 2 dates and 2 locations averaged, were as follows: Triplet and Brevor, 40; Golden, 38; Elgin, 33; Omar, 27; Elmar, 26; Kharkof, 15; Rio, 13; Burt, 7; and Columbia, 2. These data were considered inadequate for statistical treatment, but they offer encouraging prospects of control through varietal resistance.

Brevor striped strongly, and the culms were brown. When split longitudinally, the nodal areas were dark brown to black, particularly just above the cavity of the subtending internode. Brevor either died before kernel formation and had empty white heads or developed poorly filled heads.

Triplet blighted easily, and white empty heads were abundant.

Golden, a highly pigmented variety, striped strongly. Necks of healthy Golden were lavender, whereas those

of diseased Golden were dull gray.

Striped leaves of Elmar, Omar, and Elgin, all white winter club types, remained turgid and resisted withering, thus retaining the diagnostic symptoms of the disease until maturation. This characteristic resulted in their being good hosts in which to detect the disease on field trips. The straw was shortened and moderately discolored. Heads seldom blighted completely, but the kernels were shrivelled.

Stripes in Rio, Wasatch, and Kharkof (hard red winter types) were not particularly noticeable. The diseased leaves withered and collapsed readily, making the stripe symptom transient and obscure. At harvest, culms of diseased plants lacked the brilliant amber lustre of healthy straw but rather were dull and lustreless. White, blighted, empty heads were the typical symptom.

The exploratory results of this trial place Triplet, Brevor, and Golden as susceptible; Elgin, Omar, and Elmar as intermediate; Kharkof, Burt, and Rio as resistant; and Columbia as highly resistant. The spectacular, white empty heads alone are not a dependable measure of disease losses, as the white winter club wheats suffered a considerable yield loss with very few completely blighted heads. Further trials will be necessary to assess adequately varietal reactions to the stripe disease, but the performance of Columbia in this trial suggests the presence of real resistance.

A satisfactory inoculation technique is required before large-scale testing of varieties is feasible. Hypodermic inoculations are too laborious, and infection through belowground parts could conceivably give different results.

Discussion.—The genus *Cephalosporium* is usually considered as comprised mainly of saprophytic members of the soil microflora. An increasing number of them, however, are being associated with vascular disorders in plants. Fresenius, according to Nisikado et al (15), found *C. acremonium* Cda. on corn as early as 1863. Butler and Khan (6) described *C. sacchari* as the incitant of a wilt of sugarcane in 1913. In 1924, Reddy and Holbert (17) gave a detailed description of the black bundle disease of corn incited by *C. acremonium*. Vascular wilts ascribed to *Cephalosporium* spp. have been found in the dicotyledonous hosts, *Ulmus americana* L. (10), *Diospyros virginiana* L. (9), and soybeans (1). The stripe disease of wheat and the pathogen *C. gramineum* were added by the Japanese (15). These diseases establish the existence of a group of xylem-invading species within the genus *Cephalosporium*. Diverse genera of vascular-invading fungi have long been a problem in dicotyledonous plants, but to the author's knowledge the genus *Cephalosporium* is alone in containing vascular fungous parasites of the *Gramineae*.

The 3 known species of *Cephalosporium* attacking the *Gramineae* form a closely knit group. Nisikado et al (15) distinguished *C. gramineum* from *C. sacchari* on the basis of the lower temperature requirements of the wheat pathogen, and on the nature of the conidiophores. The conidiophores of *C. sacchari* have a rather definite shape, and mucilaginous spore heads were not observed (6). The conidiophores of *C. gramineum* are more variable than are those of *C. sacchari*, and spores in droplet-like heads are common. *C. acremonium* differs from the other 2 in that the conidia are smaller (13, 15), its temperature requirements are higher (13), it does not attack wheat (13), and bright colors are typical under proper environmental conditions (13, 15). The evidence warrants the maintenance of the 3 species.

Rapid internal spread by means of conidia carried in the xylem elements seems essential to explain disease development. Goss and Frink (10), in observations on elms, found that disease developed rapidly above, and slowly below, the point of inoculation. Initially, local areas of discolored vascular tissue developed; later, these areas became confluent. Allington and Chamberlain (1) demonstrated conclusively, by forcing conidia of *C. gregatum* Allington & Chamberlain through segments of healthy and diseased soybean stems, that these spores could pass through xylem elements. In wheat, Nisikado et al (15) and the author (3) have seen conidia in xylem vessels. The earliest visible symptoms on grass leaves are minute necrotic foci on the main vascular bundles; these foci later extend to become continuous with other foci. In Burt wheat, partially blighted spikes, superficially resembling spikes with the scab disease, are evidence of local internal infection. When spores in aqueous suspension were injected near the base of healthy Elmar wheat plants that were over 2 ft. tall and had headed, the fungus was recovered from rachis segments within 6 days. For this slow grower (about 1 in. per month on potato-dextrose agar at constant optimum temperature) to invade a large wheat plant in a month or so requires a means of dispersal other than direct mycelial extension. These observations leave little doubt that conidial movement within the host constitutes an important phase of pathogenesis.

Allington and Chamberlain (1) found that *C. gregatum* sporulates most profusely at temperatures below optimum for mycelial development; they suggested this may be a factor in the rapid spread of the parasite within the host in cool weather. A similar relationship between sporulation and mycelial development exists in *C. gramineum*. It appears, from its performance in culture, however, that sporulation in *C. gramineum* should be adequate for dissemination within the host at any temperature at which the fungus grows.

Toxic metabolities, in addition to plugging of vessels with mycelium, probably play a role in pathogenesis. Ikata and Kawai (12) found that *C. gramineum* in culture produces toxic substances that inhibit germination. The work of Chamberlain and McAlister (8) and of Chamberlain (7) indicates that other species of *Cephalosporium* produce toxic materials. The observation of discolored xylem and phloem in striped wheat stems well above heavily invaded portions suggests the presence of toxic materials in infected wheat, as does the generally discolored straw at maturity. Some wheats produce a crop even when infected; per-

haps these wheats tolerate toxic substances, or perhaps little toxin is produced in them.

The discovery of a virulent pathogen of wheat in an area largely dependent on that crop might be alarming. The present known wide distribution of the pathogen, especially in isolated localities such as vacant city lots and on Whidbey Island, suggests long existence in the area. The discovery of wider cultural variability among Washington isolates than was found in Japan also supports this view. The most logical conclusion is that this stripe disease is not "new" to Washington but that it had escaped detection. The ability of *Cephalosporium* spp. to survive long trips in the air (16, 18), however, offers another possibility. Windborne spores conceivably could cross an area the size of Washington in short time.

Field observations show this disease has some economic importance, with great potential for destruction (as in Japan). It is not merely a "mycological curiosity" but should be considered along with take-all, Cercosporella foot rot, and common root and crown rot in the humid and subhumid soil sites of Washington. Stripe differs quite strikingly from these better-known diseases in that the pathogen can be recovered from tissues well above the soil line.

That this pathogen may play a role in the dryland prematurity blight of central Washington (2) remains at present a hypothesis. The diseases cause similar gross pathogenic effects on certain varieties; the possible relationship to these diseases should be determined.

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CONTROLLED INOCULATION OF WHEAT SEEDLINGS WITH UREDIOSPORES OF PUCCINIA GRAMINIS VAR. TRITICI [1]

J. B. Rowell and C. R. Olien [2]

SUMMARY

Controlled inoculation of wheat seedlings with urediospores of *Puccinia graminis* var. *tritici* was accomplished with nonphytotoxic, refined, paraffinic oil carriers and an atomized spray in a special chamber. Spore deposits on seedlings in replicate pots were reproducible within a 15 per cent error and between trials on different dates within a 25 per cent error. Infection was directly related to the number of spores deposited within the range tested. Viscous oils were found to affect the infection process adversely, partly by reducing the number of appressoria and partly by the formation of many appressoria away from stomata. The amount of infection obtained from inoculation at different times was similar.

Reliable quantitative procedures for inoculating wheat plants with uniform numbers of urediospores of *Puccinia graminis* var. *tritici* (Eriks. & E. Henn.) Guyot would aid many basic investigations of wheat stem rust such as those dealing with the physiology of infection, the nature of host "field resistance," aggressiveness of biotypes, and chemical control. With present methods, urediospores are applied either by dusting or by spraying with aqueous suspensions. The cyclone duster and the use of talc as a diluent for the spores improved earlier inoculation techniques (6). Unfortunately, dusted spores clump excessively and water wets and disperses spores poorly, even with the addition of wetting agents. Dusting towers have been used to control the deposit of inoculum and to minimize clumps of spores (1), but the method seems cumbersome for inoculating large numbers of plants at frequent intervals. The compatibility of urediospores of the stem rust fungus with certain non-phytotoxic paraffinic oils indicated their possible use for precise inoculation of plants for greenhouse studies. Spores in these oils disperse easily and uniformly, germinate normally (2), and infect sprayed plants under proper conditions (3). Any oil method requires equipment that will apply light deposits to avoid the phytotoxic effects of excessive quantities of oil. A spray chamber has been designed and constructed to apply atomized oils to primary leaves of wheat seedlings at rates as low as 1 gal. per acre.

EQUIPMENT AND PROCEDURE.—The upright primary leaves of wheat seedlings are an excellent target for a horizontal spray stream. The spray chamber (Fig. 1) designed for such a spray is constructed of ¾-in. all-weather plywood made airtight with reinforced joints and calked seams. An exhaust fan and baffle plate control and direct the atomized spray pattern. The exhaust fan, a rotary twin-blade kitchen type, is mounted midway at the top of the back side. The baffle plate, mounted 1 ft. in front of the back side of the chamber, is fitted flush with the sides of the box, and the 8-in. hole is centered in relation to the spray nozzle and the seedling plants. A sliding, Plexiglass door (not illustrated) is weatherstripped to minimize drafts and provides access to the turntable. The absence of drafts and the placement of the baffle prevent lateral and vertical whipping of the atomized mist cloud. The turntable is operated by a 1/32-h.p.

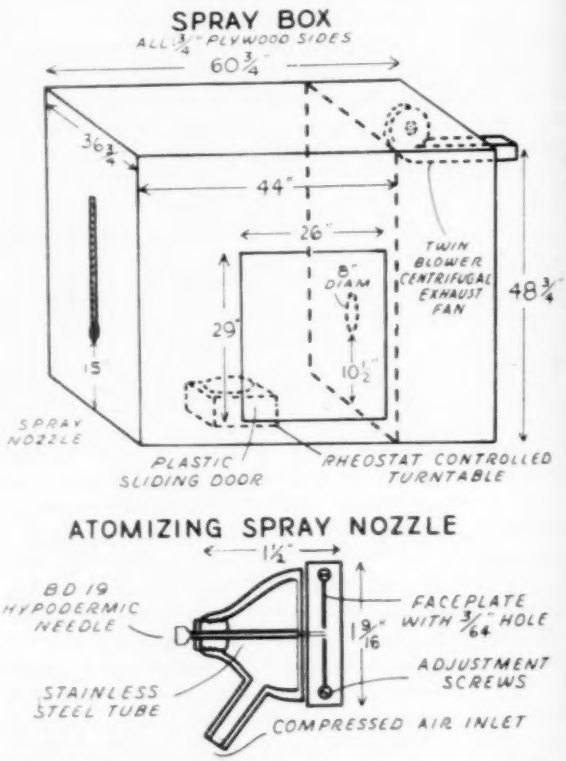

FIG. 1. Diagram of a spray chamber designed for atomization of small quantities of suspensions of urediospores of *Puccinia graminis* var. *tritici* in oil carriers.

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universal motor and its speed is controlled by a rheostat.

The atomizing nozzle was constructed from a Bean hydraulic spray nozzle and resembles a venturi in which the rate of fluid delivery and atomization is slowed by the narrow bore of the liquid delivery tube. The bell of the nozzle functions as an air chamber, with the air delivery inlet threaded into the side wall. A ¼-in. pipe plug is mounted in the nozzle inlet, and a stainless steel tube is soldered into a hole bored through its center. This tube supports and prevents vibration of the liquid delivery tube. The latter consists of a No. 19 gauge hypodermic needle cut and smoothed to fit flush in the hole of the face plate when the nozzle is assembled; the needle is soldered to the support tube. Three setscrews in the cover ring of the spray nozzle are used to adjust and center the hole of the face plate around the end of the fluid delivery needle. The spray nozzle is mounted on an adjustable support in a 1 × 8-in. vertical slot in the chamber wall opposite the baffle and exhaust fan. The spray nozzle is aligned with a point light-source mounted on the wall opposite the hole of the baffle plate and in a position corresponding to the center of the plant surfaces. The nozzle position is checked at any time by sighting through the fluid delivery tube on the point of light. Air for the nozzle is supplied by a small tank air compressor at 10 lb./sq. in. and is controlled by a hand operated cut-off valve.

Wheat seedlings to be sprayed are grown in 4-in. pots. Ten seeds per pot are sown with a circular template, and the seedlings are thinned to 5 uniform plants with fully extended primary leaves in a pentagon pattern. The pot of seedlings to be sprayed is centered on the turntable and its rotation is adjusted to 80 rpm. The air valve is opened, and 0.1 ml of the spore suspension, usually containing 1 or 2 mg of urediospores per ml in a 50:50 oil mixture of Socony Vacuum Mobilsol 100 and U.S.P. light petrolatum, is delivered by a micropipette into the cup of the hypodermic needle. Approximately 15 seconds are required for the atomization of this amount of oil, and when completed, the plants are replaced with another set. Two operators can inoculate 36 pots in 30 minutes by this procedure.

The standard procedure used for infection conditions is based on a study (4) of the effect of environment on the infection process of urediospores of *P. graminis* var. *tritici*. Plants are inoculated in the spray chamber between 8:30 and 9:00 a.m., then atomized with distilled water and placed in a constant-temperature (70°F ± 1.5°) dew chamber. At 5:00 p.m., the plants are removed to the floor of a constant-environment room (65°F) inside open chambers covered with a double layer of wet cheesecloth. This procedure permits the plants to dry slowly in indirect light so that the appressoria remain quiescent, although the light period of this room is from 5:00 p.m. until 5:00 a.m. with the relative humidity ranging from 40 to 50 per cent. The plants are moved at 8:00 a.m. on the second day after inoculation to the greenhouse, where the final stages of infection occur.

CALIBRATION OF SPRAY DEPOSIT.—Spray pattern and deposit characteristics were determined with a saturated solution of Dupanol Red, an oil-soluble dye, in the 50:50 oil mixture. The densest portion of the spray pattern in the chamber was established to be 16 cm in diameter by spraying an upright frame of ⅛-in. glass tubing with dye solution. The fully extended primary leaves of wheat seedlings used in spray tests averaged 9–10 cm in length and 0.45 cm in width, and the pattern was centered on the seedlings. Originally, a position 15 in. from the spray nozzle was selected for the spraying position. The amount of oil deposit was determined by spraying 0.1 ml of oil saturated with dye onto a glass microscope slide mounted vertically on the turntable at plant height. The deposit on the slide was washed with acetone into a 10-ml volumetric flask and made to volume with this solvent. The optical density of the acetone solution washed from the slide was determined with a Coleman Junior Spectrophotometer at a wavelength of 4050A and the readings were converted to μl of oil per cm^2 by interpolation from a graph prepared from determinations on a series of dilutions in acetone of the original oil-dye solution. Atomization of 0.1 ml of oil-dye solution resulted in a mean deposit of 0.065 μl/cm^2 on glass slides. Variation between replicate slides was within an error of 10 per cent, and the deposit was duplicated within a 10 per cent error in determinations repeated on different days. Primary leaves of wheat seedlings were also sprayed with 0.1 ml of the oil-dye solution and the deposit washed off immediately with acetone as before. A deposit of 0.072 μl/cm^2 was obtained for this material, but adsorption of the dye was an unknown factor. Blanks of acetone washes from unsprayed leaves had negligible optical density, indicating that the readings obtained for oil-dye deposits were not influenced by materials washed from the leaves by acetone.

The number of spores per unit of surface deposited by the equipment agreed with the deposit of oil determined spectrophotometrically. In these determinations, 2 slides mounted back to back were sprayed with 0.1 ml of a suspension of urediospores in the oil mixture, and counts were made of the number of spores in 6 strips 1 field wide and 1 cm apart across each of the slides (3.5 cm^2 per pair of slides). The number of spores in the original spore suspension was determined with a haemocytometer. The amount of oil/cm^2 on the slide surfaces was calculated by dividing the number of spores/cm^2 on the slide surface by the number of spores/μl in the original spore suspension. The mean counts for 6 pairs of slides gave an oil deposit of 0.060 μl in one determination and one of 0.058 μl/cm^2 in a second. This method has been used routinely to check the performance of the spray equipment during inoculation trials; a pair of slides was sprayed at the start and at the completion

TABLE 1.—*Deposit of urediospores of the stem rust fungus on primary leaves of wheat seedlings and on glass slides sprayed with an oil suspension in a controlled inoculation chamber*

Date of trial (1956)	Urediospore suspension Spores/ml	Deposit on leaves		Deposit on slides		Ratio: leaves / slides
		Spores/cm²	Oil μl/cm²	Spores/cm²	Oil μl/cm²	
	($\times 10^6$)					
Aug. 20	0.54	22.1	0.041	13.2	0.026	1.67
23	0.92	33.1	0.036	24.2	0.026	1.38
29	1.1	32.1	0.029	19.9	0.018	1.67
30	0.555	23.3	0.042	14.4	0.026	1.62
31	0.61	19.6	0.032	13.6	0.022	1.45
Sept. 6	1.77	78.9	0.045	42.7	0.024	1.87
Nov. 2	1.89	89.7	0.047	53.4	0.028	1.68
Mean			0.039		0.024	1.62

of each series. The mean oil deposit for 22 inoculation trials was 0.061 μl/cm².

A second position 20 in. from the spray nozzle was also calibrated. A mean deposit rate of 0.037 μl/cm² was obtained with the oil-dye solution for glass slides in this position. A mean deposit rate of 0.039 μl/cm² was obtained for urediospore counts on slides in 12 inoculation trials.

The numbers of spores deposited on leaves also were counted. The spray nozzle had been cleaned and readjusted prior to these determinations, and on recalibration by the dye method a deposit of 0.023 μl/cm² was found for the 20-in. position because of finer atomization of the spray. Deposits on plant surfaces were determined by clipping the 5 leaves from each of 3 pots immediately after they were sprayed and mounting the leaves on microscope slides by narrow strips of Scotch tape at each end. Counts of spores on the undisturbed surface of each leaf were made in a strip approximately 6 cm long (the precise length of the strip scanned was determined with the stage micrometer) and 1 field wide. Counts were also made by the previously outlined procedure for the deposit on duplicate pairs of slides. The results are summarized in Table 1. The mean deposit of spores obtained for 7 separate trials was 0.039 μl/cm² for leaves and 0.024 μl/cm² for slides, indicating a 62 per cent greater deposit on leaf surfaces than on slides. The increased deposition on leaves was ascribed to the differences in deflection of the spray pattern by the wide surface of slides as compared to the leaves. The deposit rates for replicate pots were within ± 15 per cent of the mean.

The gallons-per-acre equivalents of these deposits have been estimated. The surface area calculated from the data of van de Sande-Bakhuyzen (5) for the standard wheat plant (variety Hard Federation) is 205.8 cm². At a seeding rate of 300 ear-bearing straws per sq. yd., there would be 2.91×10^8 cm² of plant surface per acre. Complete coverage of this plant surface by 1 gal. of liquid would result in 0.013 μl per cm². Thus, a deposit of 0.039 μl/cm² on leaves of seedling wheat sprayed in the chamber is equivalent to the ideal coverage of an acre of wheat by 3 gal. of liquid.

RELATION BETWEEN SPORE DEPOSIT AND AMOUNT OF INFECTION.—The amount of infection was found to be directly proportional to the deposit of spores under a variety of environmental conditions. Repeated inoculation trials were made with a series of dilutions of spores in the 50:50 oil mixture. A typical trial, made with the original nozzle arrangement and the 15-in. spray position, is illustrated in Figure 2. Five replicate pots of seedling wheat (Little Club) were inoculated as previously described with 0.1 ml of each of a series of dilutions of spores (race 15B) suspended in the oil mixture. Duplicate pairs of slides were also sprayed with the various spore dilutions, and the spore deposit was counted as outlined previously. The concentration of spores in the dilution series was determined by triplicate haemocytometer counts. Calculation gave a mean deposit for slide surfaces of 0.060 μl/cm² within a range of 10 per cent. The plants were exposed to a 23-hour dew period at 70.5°F ± 1.5° in the dew chamber, then removed to a greenhouse where the plant surfaces dried rapidly. The number of pustules per leaf were counted 10 days later. The mean amount of infection was 0.24 uredia

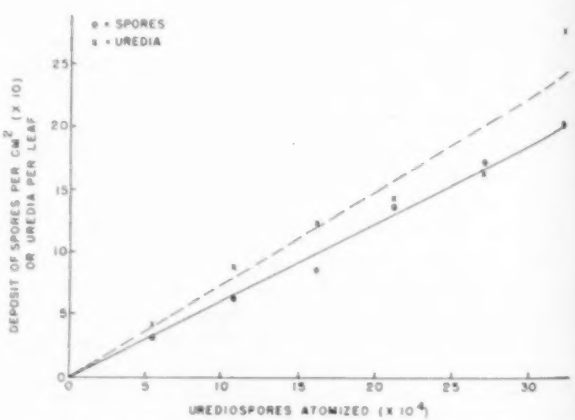

FIG. 2. Relation between numbers of spores of *Puccinia graminis* var. *tritici* in the volume of oil suspension atomized, the spore deposit on leaves, and the number of uredia per leaf in tests in which a controlled inoculation method was used.

per 100 spores in this experiment. The low rate of infection was found subsequently (4) to be due to the inadequacy of the infection environment. The linear relation for spore deposit and amount of infection indicate the direct relation between these processes and the reliability of the inoculation method.

EFFECT OF OIL ON SPORE DEPOSIT AND AMOUNT OF INFECTION.—The 50:50 oil mixture has certain adverse effects on infection. Few stem infections developed from oil inoculations in field tests (3). Plants inoculated with urediospores in oil in the greenhouse were noted to fleck approximately 1 day later than did plants dusted with spores, and although differences in pustule development disappeared in susceptible host-race combinations within a few days, in resistant combinations the disease reaction was weak for the oil-inoculated treatments. The 50:50 oil mixture was originally selected because it had certain manipulative advantages over the individual component oils. Mobilsol 100 oil is relatively light in viscosity and in specific gravity, hence it atomizes rapidly through the spray nozzle and suspended spores settle rapidly during the pipetting operation, rendering control over deposit difficult. Furthermore, the oil spreads rapidly on the counting surface of the haemocytometer, making reproducible counts exceedingly difficult. In contrast, the heavy viscosity of the U.S.P. light petrolatum oil made difficult the initial suspension of the spores, although the spores remained dispersed for longer periods than they did in the lighter oil, and the oil had to be forced from the pipette into the atomizing tube. Thus, the 50:50 oil mixture was a compromise between the advantages and disadvantages of the 2 oils.

A distinct effect of the 50:50 oil mixture on appressoria formation was observed during studies (4) of the effect of environment on the infection of wheat seedlings by urediospores of the stem rust fungus. Epidermal peels were examined by microscope at regular intervals during these studies to determine the percentage of spore germination, appressoria formation, and substomatal vesicle formation. A considerable number of the appressoria were not formed over stomata but in such locations as the indentations of the epidermis over small veins or at the junction of epidermal cells. Comparative counts were made of plants inoculated with dusted and oil-carried urediospores. Although the same percentage of spores (94 per cent) germinated for oil-carried and for dusted inocula, the percentages of appressoria over stomates were 48 and 85 per cent, respectively. None of the appressoria in nonstomatal locations were ever observed to penetrate the epidermis and produce vesicles, therefore these misplaced structures were presumed to be noninfective. The effect of oil on appressorial location apparently was a major factor affecting the amount of infection obtained in all infection trials.

The tendency of germ tubes of urediospores carried in oil to form appressoria in surface irregularities can be readily demonstrated *in vitro*. Strips of polyethylene (1 mil thick) were scratched lightly with fine wire insect-mounting pins and mounted on frames. Similar unscratched strips were also mounted on frames. The strips were sprayed with spores in oil, moistened with a spray of distilled water, and placed in a dew chamber for 8 hours. The strips were removed from the frame, mounted on slides in acidified cotton blue, and examined. Only a trace (less than 1 per cent) of the germ tubes formed appressoria on the unscratched polyethylene strips, whereas appressoria were formed abundantly in the scratches; the frequency varied with the number of scratches.

The influence of appressorial location on amounts of infection for inocula carried in oil was demonstrated by inoculations with the individual component oils. A suspension of 5 mg of urediospores (race 15B) in 5 ml of the mixture and of each of the 2 component oils (Mobilsol 100 and U.S.P. light petrolatum) was sprayed on 5 replicate pots of seedlings (Little Club) for each treatment and then exposed to the standard conditions for infection. Data for spore deposit, uredia per 100 spores, and appressorial characteristics are listed in Table 2. The increase in the amount of deposit with increasing viscosity for the 3 oils was confirmed by spectrophotometric determinations with the oil-soluble dye. The $\mu l/cm^2$ deposit for 0.1 ml of atomized oil was 0.017, 0.023, and 0.044 for Mobilsol 100, the 50:50 oil mixture, and U.S.P. light petrolatum, respectively. Conversely, both the total number of appressoria and the number formed over stomata decreased with an increase in the viscosity of the carrier oil. Similarly, a decrease in infection reflected the adverse effect of oil viscosity on appressorial development. In addition to this effect, the more viscous oils are believed to have an adverse effect on the later stages in the infection process, since

TABLE 2.—*Amount of infection by Puccinia graminis var. tritici and characteristics of appressoria in inoculation trials with urediospores carried in 3 oils*

Oil	Trial I		Trial II				
						Appressoria	
	Spore deposit (per cm^2)	Uredia per 100 spores	Spore deposit (per cm^2)	Uredia per 100 spores	Germination (%)	Per 100 germ tubes	Stomatal (%)
Mobilsol 100	13.3	4.32	10.8	8.18	86	49.7	84
50:50 oil mixture	20.3	0.75	19.8	2.96	85	39.0	47
U.S.P. light petrolatum	32.1	<0.05	28.0	0.335	87	28.7	27

the proportionate decrease in numbers of uredia among the 3 oils was greater than the differences in appressorial characteristics.

A series of comparative tests was made with 23 different spray oils from various commercial companies. The constant environment facilities were inadequate for testing all of these oils simultaneously, therefore groups of 6 oils were compared with the 50:50 oil mixture in each trial. Equal amounts of spores were dispersed in each of the oil samples (10.0 mg/5 ml of oil), 4 replicate pots of wheat seedlings and duplicate slide pairs were sprayed with each of the spore suspensions (0.1 ml in 20 seconds per pot), and the standard procedure was followed for infection. Duplicate drops of each spore suspension were placed on 1 per cent water agar and counts were made 24 hours later to determine germination behavior. No differences were observed in the amounts of germination in the various oils, but some oils caused abnormal germ-tube development. Data for deposit and infection from the various comparative trials is expressed as the ratio between each oil and the 50:50 oil mixture in Table 3. Deposit ratios indicate the relative viscosity of the oil. Those oils with a ratio greater than 1 were more viscous than the 50:50 oil mixture used as a standard and all of this group had an infection ratio of less than 0.5. Seven of the 10 less viscous oils had infection ratios greater than 0.5. The other 3 affected the character of germ tube growth adversely. The deposit of all of the less viscous oils evaporated from the glass slides within an hour after spraying. The 5 oils (Mobilsol 100, Bayol D, L8779, L2687, and Ortho SOS-B) that resulted in considerably better infection than did the 50:50 oil mixture were among the lightest oils tested, and the characteristics of rapid volatility, rapid atomization, and rapid spore settling were unfavorable for controlled inoculation trials. The poorer infection with the more viscous oils apparently results from the adverse effects on the development of appressoria and on later stages of infection. The urediospores in the light oils with the best infection probably behave normally.

TABLE 3.—*Comparative effectiveness of various oils as carriers for inoculating wheat seedlings with urediospores of the stem rust fungus*

Company	Oil designation	Ratio to standard mixture[a]	
		Urediospores per cm²	Uredia per 100 spores
California Spray Chemical Corp.	Ortho SOS-H	2.1	0.15
Gulf Oil Corp.	530	1.8	0.099
Gulf Oil Corp.	372	1.7	0
Pennsylvania Refining Co.	Drakeol 10	1.7	0.025
Gulf Oil Corp.	303	1.7	0.041
Gulf Oil Corp.	503	1.6	0.077
Sherwood Petrolatum Co.	U.S.P. light petrolatum	1.5	0.088
Pennsylvania Refining Co.	4462	1.5	0.098
California Spray Chemical Corp.	Ortho SOS-L	1.4	0
California Spray Chemical Corp.	Ortho SOS-K	1.4	0.062
California Spray Chemical Corp.	J-16	1.3	0.26
Esso Standard Oil Co.	Bayol 85	1.3	0.023
Esso Standard Oil Co.	Marcol JX	1.3	0.14
Gulf Oil Corp.	371	1.2	0
California Spray Chemical Corp.	Mineral Seal Oil	0.9	0.8
Esso Standard Oil Co.	Mentor 28	0.9	0.12[b]
Socony Vacuum Oil Co.	Mobilsol 100	0.8	3.0
Standard Oil Co. (Indiana)	L6942	0.7	0.092[b]
Esso Standard Oil Co.	Bayol D	0.7	5.9
Standard Oil Co. (Indiana)	L8770	0.6	11.2
Standard Oil Co. (Indiana)	L1543	0.6	0.22[b]
Sinclair Refining Co.	Odorless base	0.6	0.53[b]
Standard Oil Co. (Indiana)	L2687	0.5	10.5
California Spray Chemical Corp.	Ortho SOS-B	0.5	2.54

[a] A 50:50 mixture of Mobilsol 100 and U.S.P. light petrolatum.
[b] Oils in which spores germinated abnormally.

REPRODUCIBILITY OF AMOUNT OF INFECTION.—The amount of infection obtained by the described method has been controlled reasonably well in repeated trials. Data in Table 4 illustrate the amounts of infection encountered when this procedure was used over a period of time with spores carried in the 50:50 oil mixture and in Mobilsol 100. Differences between the numbers of uredia per leaf (and per 100 spores) on different dates reflect variation in deposit and in spore mass in different seasons. A steady decline (with time) in the amount of infection obtained with spores carried in the 50:50 oil mixture coincided with the seasonal diminution of light intensity and duration in the last $\frac{1}{3}$ of 1956. Mobilsol 100 was substituted for the mixture following the discovery of the better infection with the less viscous oils. The reproducibility

TABLE 4.—*Reproducibility of amount of infection by Puccinia graminis var. tritici on primary leaves of wheat seedlings inoculated in a spray chamber with oil-carried urediospores and infected under a standardized environmental procedure*

Mixture of Mobilsol 100 and mineral oil 1 mg spores/ml			Mobilsol 100 2 mg spores/ml		
Date of trial	Uredia per leaf	Uredia per 100 spores	Date of trial	Uredia per leaf	Uredia per 100 spores
8/29/56	21.2	17.3	10/31/56	29.4	10.5
8/31	16.1	11.4	12/26	19.8	8.2
9/12	13.9	10.5	12/27	20.9	8.1
10/4	23.2	12.3	12/28	14.1	5.0
10/16	13.6	8.1	1/2/57	17.2	5.3
10/23	14.2	8.2	1/8	16.6	5.6
10/31	14.1	7.6	1/15	19.3	8.3
11/7	9.1	5.1	1/17	17.2	7.4
11/14	9.2	5.6	1/19	18.7	8.9
11/21	9.5	4.5	1/22	11.1	5.8
12/26	6.5	3.0	1/24	12.5	4.7

in the amounts of infection between separate trials is within a reasonable range for a variety of experimental purposes. It is believed that constant environmental facilities for all stages of plant and disease development would be required for further refinement in the method.

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EFFECT OF SOME SOUR CHERRY VIRUSES ON GROWTH OF YOUNG ORCHARD TREES [1]

J. A. Milbrath

SUMMARY

Montmorency sour cherry trees were inoculated while in the nursery row with various combinations of mild and severe strains of ring spot, sour cherry yellows, peach stunt, prune dwarf, a sour cherry bark-splitter, and sour cherry midleaf necrosis viruses. When any of these viruses was present, a substantial reduction in growth was noted when tree measurements were made 4 years later. This reduction in growth increased with strain severity and with the number of viruses present. Four-year-old infected trees varied in height from 1 to 4 ft. and also in tree diameter from 0.9 to 2.8 in.

A planting of 107 Montmorency sour cherry trees (*Prunus cerasus* L.) was established for use in an attempt to separate the ring spot and yellows virus components of the sour cherry yellows disease. All of these trees were progeny of Oregon's clonal line of the variety Montmorency 505 and were propagated on mazzard roots. Seventy-seven were progeny of trees that had been inoculated with virus in buds from a Shirofugen filter tree that had been used to separate the midleaf necrosis virus from those causing ring spot and sour cherry yellows (5). Forty-four of these trees developed midleaf necrosis symptoms; 33 failed to develop foliage symptoms but did not make normal growth, an indication that some virus was present. The other 30 trees involved in this experiment were propagated from a virus-free check tree. While the trees were still in the nursery row, they were inoculated with various virus combinations from 7 sources (Table 1).

The trees were removed from the nursery row the spring after inoculation and established 10 ft. apart in each direction in an experimental planting. Four years later, the differences in growth due to the effect of the different virus combinations in them was so apparent (Fig. 1) that records were made on trunk diameter, tree height, and diameter of the tree in the area of greatest branch spread. Data were recorded for 3 classes: 1) trees that were virus-free before inoculation, 2) trees that were progenies from those involved in the isolation of midleaf necrosis virus but that did not develop leaf symptoms, and 3) trees from the same inoculated mother trees as group 2 but showing midleaf necrosis.

The data for the Montmorency trees that were virus free when inoculated with the various virus combinations follow a logical descending trend surprisingly well for 2-tree replications (Table 2). The noninoculated checks compared well with the 2 trees budded from the virus-free Bing tree. RS 2, which carried only the mild bark-splitter virus (1), reduced growth only slightly. The trees inoculated with mild strains of peach ring spot virus (RS 4 or RS 5) were only slightly

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TABLE 1.—*Index history of the trees used as source of virus*

Source	Reaction[a] on test plants indicated					
	Shiro-fugen	Kwanzan	Prune	Cucumber	Montmorency	Peach
RS 1[b]	—	—	—	—	—	—
RS 2	—	—	—	—	SCBS	—
RS 3	Sy-N	M	D	S	SCY	PRS-St
RS 4	Sy	S	—	—	SCBS	—
RS 5	N	—	—	M	—	—
RS 8	N	S	D	S	SCY-SCMN	PRS-St
RS 9	N	S	—	S	SCBS	PRS
RS 10	N	S	D	S	SCY-SCMN	PRS-St

[a] Symptoms on diagnostic hosts for peach ring spot virus indicated as follows: — = no reaction, Sy = systemic reaction, N = necrotic reaction, M = moderate reaction, and S = severe reaction. Virus indicated by other diagnostic hosts as follows: SCBS = sour cherry bark-splitter virus, SCY = sour cherry yellows virus, SCMN = sour cherry midleaf necrosis virus, PRS = peach ring spot virus, D = prune dwarf virus, and St = peach stunt virus.

[b] Virus-free Bing (B260) used as a mechanical check.

less vigorous than the foregoing, but those inoculated from RS 3, RS 8, or RS 10, which contained rather severe strains of peach ring spot virus as well as other virus components, were markedly stunted.

Of special interest in these experiments was the unknown entity or virus present in some of the trees when inoculated. This factor caused no definite foliage symptom, but trees containing it grew less than did virus-free trees (Table 2). Since the trees infected with this "unknown" virus originally came from the same trees as those expressing midleaf necrosis, it could be a very mild strain of this virus. If this is not the case, it must be considered an unidentified latent virus. The reduction of growth from this factor was striking, and the measurements show a reduction in over-all growth that was readily apparent (Fig. 1, B). When other viruses were present, the effect of this virus on growth was still evident.

The trees that expressed symptoms of midleaf necrosis (Table 2, C, item 1; Fig. 1, C) showed the most striking reduction in growth. Even when no other virus was present, some of the trees were nearly 3 ft. shorter than the virus-free trees (Table 2, A, item 1; Fig. 1, A). When these trees were also infected with the more severe viruses in the complex, they made still less growth; such trees would be of no practical value in a commercial orchard planting.

The possibility of a contamination with the K and S little cherry virus (10) has not been eliminated, hence the "unknown" might be K and S virus; how-

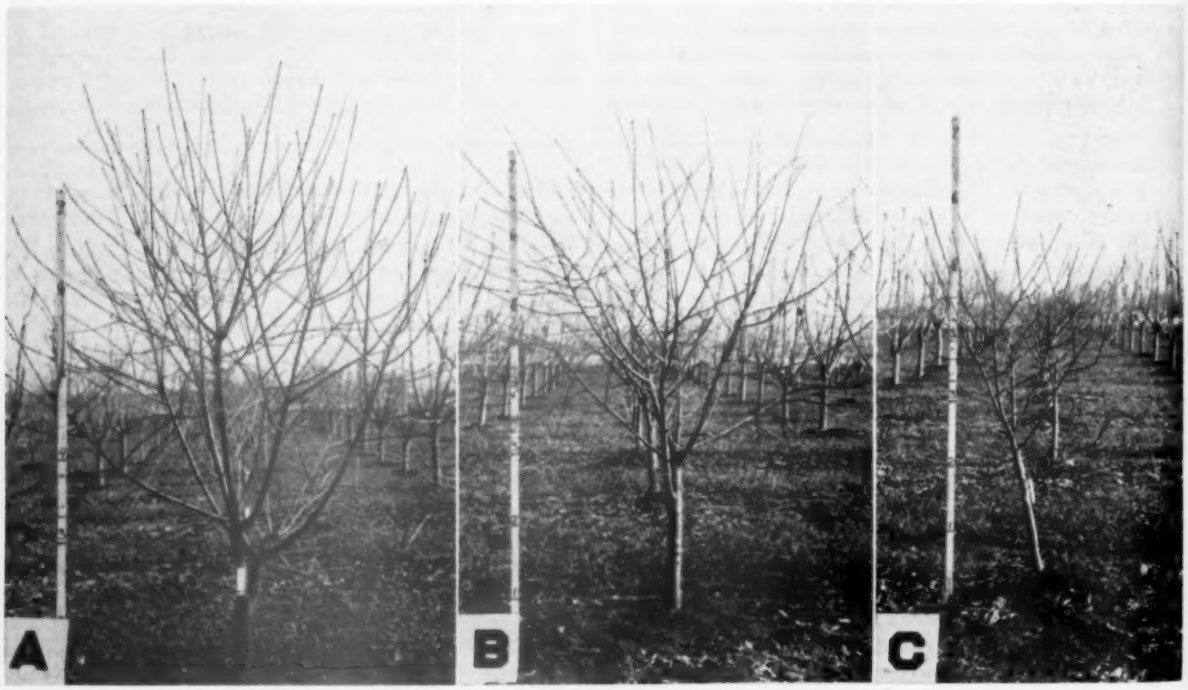

FIG. 1. Four-year-old Montmorency trees showing the effect of viruses on the growth and development of the trees. A) A normal virus-free tree; B) Tree propagated from bud infected with an unidentified virus; C) Tree propagated from a bud infected with midleaf necrosis virus and possibly with the unknown virus. (Photograph by H. H. Millsap).

TABLE 2.—*Measurements of Montmorency sour cherry trees infected by various viruses or virus combinations and measured 4 years after inoculation*

Inoculum		No. of trees	Mean trunk diameter (in.)	Mean height of trees (ft.)	Mean Diameter of trees [b] (ft.)
Source	Viruses present [a]				
	(A. Trees virus free before inoculation)				
None	None	14	2.8	8.8	8.6
RS 1	None	2	2.8	8.5	9.0
RS 2	SCBS	2	2.6	8.5	7.8
RS 5	PRS	2	2.4	8.5	8.3
RS 4	PRS-SCBS	2	2.0	7.0	5.6
RS 9	PRS-SCBS	2	2.0	6.5	6.0
RS 3	PRS-SCY	2	1.9	6.6	6.8
RS 8	PRS-SCY-SCMN	2	1.8	6.0	5.5
RS 10	PRS-SCY-SCMN	2	1.3	6.3	5.7
Mean		30	2.2	7.4	7.0
	(B. Trees infected with an unknown virus when inoculated)				
None	None	14	2.3	7.2	7.2
RS 1	None	4	2.8	7.5	7.5
RS 2	SCBS	5	2.1	6.4	6.1
RS 5	PRS	4	2.1	6.6	6.2
RS 4	PRS-SCBS	2	1.8	6.0	6.0
RS 9	PRS-SCBS	4	1.6	5.7	5.5
RS 3	PRS-SCY	3	1.3	5.1	4.3
Mean		33	2.0	6.3	6.1
	(C. Trees infected by midleaf necrosis virus and possibly unknown virus when inoculated)				
None	None	16	1.8	6.1	5.6
RS 1	None	2	1.6	6.5	6.0
RS 2	SCBS	3	2.3	6.8	6.4
RS 5	PRS	4	1.8	6.1	5.6
RS 4	PRS-SCBS	6	1.5	5.8	5.2
RS 9	PRS-SCBS	4	1.4	5.3	4.4
RS 3	PRS-SCY	5	1.3	5.2	4.3
RS 8	PRS-SCY-SCMN	2	0.9	4.2	3.5
RS 10	PRS-SCY-SCMN	7	1.3	5.4	4.8
Mean		44	1.5	5.7	5.1

[a] SCBS = sour cherry bark-splitter virus, PRS = peach ring spot virus, SCY = sour cherry yellows virus, SCMN = sour cherry midleaf necrosis virus.

[b] Measurement at area of greatest branch spread.

ever, neither the original trees that were inoculated directly from the Shiro-fugen filter tree infected with K and S virus nor their progeny have shown the fruit symptoms on Montmorency associated with this little cherry disease (6).

Various papers have been published that have reported a reduction in the growth of Montmorency cherry in nursery-row trees (2, 3, 4, 7, 8, 9). This reduction was assumed to be due to ring spot and sour cherry yellows. The data presented here indicate that other viruses could have been involved.

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INFLUENCE OF TEMPERATURE AND MOISTURE ON DAMPING-OFF OF AMERICAN AND SIBERIAN ELM, BLACK LOCUST, AND DESERTWILLOW [1]

Ernest Wright

SUMMARY

In greenhouse tests, pre-emergence and post-emergence damping-off was somewhat more severe in very wet soil (95 per cent of moisture-holding capacity) than in drier soil (50 per cent of moisture-holding capacity). Final stands, however, were not generally significantly less on the wet soil. Isolations from infected seedlings indicated that black locust is highly susceptible to *Pythium ultimum* at both moisture levels, whereas desertwillow proved particularly susceptible to *Rhizoctonia solani*. *Fusarium* spp. were most abundant in the drier soil.

Temperature tests showed that pre-emergence losses for American elm and desertwillow may be encouraged by low soil temperature (15° C). Rhizoctonia appeared to be more pathogenic than was Pythium at the highest soil temperature used (29° C). Temperature likewise had a direct effect on seed germination and seedling height growth. High temperature stunted American elm and stimulated desertwillow seedlings. No consistent relation was observed between the growth rates of the damping-off parasites *in vitro* and apparent virulence at the same temperatures in damping-off tests.

The present paper contains previously unpublished results of greenhouse tests made in the late 1930's on the influence of soil moisture and of soil temperature on the damping-off of 4 important broadleaf tree species. The published information gained from this study was used in the control of damping-off of broadleaf species grown in Great Plains federal shelterbelt nurseries (9).

MATERIALS AND METHODS.—The broadleaf species tested were American elm (*Ulmus americana* L.), Siberian elm (*U. pumila* L.), black locust (*Robinia pseudoacacia* L.), and desertwillow (*Chilopsis linearis* (Cav.) Sweet), all of which were found to be susceptible to damping-off (8).

The seed used in these tests was obtained through the courtesy of the U. S. Forest Service. All seed used in greenhouse tests was kept in tight containers in refrigeration at 35°–38° F.

Elm seed was dewinged before sowing, whereas black locust seed was soaked in concentrated sulfuric acid for 1 hour and then thoroughly washed prior to sowing.

Greenhouse tests were made with broadcast-sown seed in unsterilized soil placed in glazed earthenware crocks 6 in. in diameter by 7 in. deep. In the soil-moisture tests, the crocks were arranged at random on an open bench during the day. At night they were protected by a fiberboard incubation chamber with a muslin top (Fig. 1). A relative humidity of 60–70 per cent was maintained at night at 70° F. During the day, however, air temperatures sometimes rose to 100° F and humidity dropped accordingly.

For controlled temperature tests, duplicate double-walled glass chambers were used. These were kept in a cold room and the desired temperatures were obtained by regulated thermostats (Fig. 2).

SOIL MOISTURE AND DAMPING-OFF.—Hartley (3) reported that the incidence of damping-off in pine seed-

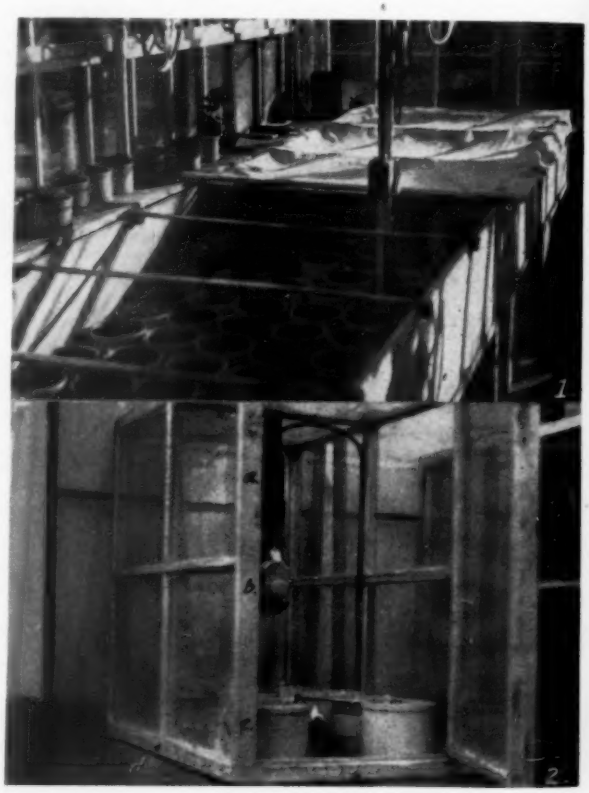

FIG. 1-2.—Fig. 1. Incubation chamber, showing portable walls in place and removable muslin top used to cover the crocks at night in order to maintain a high relative humidity.—Fig. 2. Interior of double-glass-walled temperature case. A.) Maximum-minimum thermometer for recording air temperatures. B.) Thermostat control unit. C.) Blower-type heater. D.) Water tank below case used to help maintain constant temperature and high humidity.

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TABLE 1.—*Damping-off of 4 broadleaf tree seedlings[a] grown in the greenhouse at 22°–27° C in Marshall silt loam soil maintained at 2 different moisture levels*

Species	No. seedlings emerged		Pre-emergence loss[b] (%)	Post-emergence loss[c] (%)	Final stand[d] (%)
	Sterile sand	Infested soil			
	(Soil with moisture at 50% of its moisture-holding capacity)				
American elm	592	200	66	24	20
Siberian elm	544	75	87	30	6
Black locust	568	241	58	23	25
Desertwillow	600	372	39	22	39
	(Soil with moisture at 95% of its moisture-holding capacity)				
American elm	592	103	82	39	7
Siberian elm	544	85	84	28	6
Black locust	568	181	68	62	13
Desertwillow	600	309	48	49	19

[a] Based on 8-crock replications, each planted with 100 seed.
[b] Based on number emerged in sterilized sand.
[c] Percentage of seedlings damping-off in infested soil.
[d] Percentage of seedlings based on sterilized sand.

lings grown in sandy soil decreased after heavy rains. Henson et al (2) found that excessive watering did not materially increase damping-off in coniferous seedbeds. For broadleaf seedlings, field observations during the course of this study at 3 different nurseries had indicated that damping-off is not increased by flooding with river or irrigation water. More positive evidence was needed, however.

Soil moisture tests.—The effect of soil moisture on damping-off of 4 broadleaf species was tested in the greenhouse. Two different levels of soil moisture were used (50 and 95 per cent moisture-holding capacity).

Pre-emergence losses, with the exception of Siberian elm, were numercially greater in the wet soil series than in the dry series (Table 1), but these differences were not statistically significant. Also, postemergence damping-off was mostly not significantly greater in the wetter of the 2 soils. The same trend was reflected in the final stands. These limited data agree with field observations that high soil moisture generally does not enhance damping-off of the broadleaf species tested.

Soil moisture and infection.—The influence of soil moisture on the prevalence of damping-off and other fungi was determined by isolations (Table 2).

Pythium ultimum Trow proved considerably more destructive to all 4 species of broadleaves in the wetter soil than in the drier soil. The total isolates of *Rhizoctonia solani* Kühn were also more numerous in the wetter than in the drier soil but this was due mainly to the heavy postemergence damping-off of desertwillow. It is also of interest that *Fusarium* spp. were isolated almost twice as frequently from the seedlings growing in the drier soil than from those in the wetter soil.

These data indicate that of the broadleaf species tested black locust proved particularly susceptible to Pythium, whereas desertwillow was particularly susceptible to Rhizoctonia.

SOIL TEMPERATURE AND DAMPING-OFF.—The influence of soil temperature on the development of plant diseases has been the subject of numerous investigations. Pythium has been reported to be capable of causing damping-off over a wider temperature range than is Rhizoctonia (1, 5, 6).

Hartley (4) stated that relatively high temperatures increased damping-off of coniferous seedlings infected by *R. solani* or by *Pythium debaryanum* Hesse. Roth and Riker (7) report similar findings for red pine (*Pinus resinosa* Ait.) in Wisconsin tests. In Germany, Roth (6) found that these pathogens caused most severe damping-off between 18° and 30° C. Above this range, *Corticium vagum* Berk. & Curt. (syn. *R. solani*) became entirely innocuous whereas *P. debaryanum* was still pathogenic at 33°.

Soil temperature tests.—The results of soil temperature tests for 4 broadleaf species in specially controlled temperature chambers are summarized in Table 3.

For the broadleaf tree species tested, best emergence was obtained at 29° C, except for American elm. Postemergence damping-off was also generally greatest at the higher soil temperatures, whereas pre-emergence losses showed the opposite trend. American elm germinated best at a soil temperature of 25°, Siberian elm at 15°, black locust at 20°, and desertwillow at 29°.

TABLE 2.—*Fungi cultured from 4 broadleaf tree species that were damping-off in moist and relatively wet soil*

Species	Total no. of cultures made	Percentage of isolations from diseased seedlings					
		Rhizoctonia solani	*Pythium ultimum*	Miscellaneous Phycomycetes	*Fusarium* spp.	Bacteria	Miscellaneous
		(Marshall silt loam soil with moisture content at 50% of moisture-holding capacity)					
American elm	60	15	18	5	25	8	28
Siberian elm	45	18	4	4	27	7	40
Black locust	100	0	32	12	18	30	8
Desertwillow	95	23	13	5	17	20	22
		(Marshall silt loam soil with moisture content at 95% of moisture-holding capacity)					
American elm	42	5	48	10	14	5	19
Siberian elm	26	4	31	23	19	8	15
Black locust	80	4	49	19	10	15	4
Desertwillow	139	28	23	7	14	3	24

TABLE 3.—*Seed germination and damping-off in a 3-week test period* [a] *in which seed of 4 broadleaf tree species were sown in sterile sand and in infested soil maintained at different temperatures*

Species	Average soil temperature (°C)	Total emergence in sterile quartz sand		Percentage emergence and stand in unsterilized Marshall silt loam soil			
		No.	Percentage	Total emergence	Pre-emergence loss [b]	Postemergence loss [c]	Final stand
American elm	15	80	31	61	39	14	53
	20	242	94	31	69	40	19
	25	259	100	37	63	32	25
	29	251	97	38	42	37	25
Siberian elm	15	337	100	20	80	57	9
	20	325	96	15	85	38	9
	25	318	94	19	81	73	5
	29	255	76	56	44	43	32
Black locust	15	199	63	68	32	39	41
	20	318	100	53	47	58	22
	25	303	95	63	37	55	28
	29	302	95	89	11	44	50
Desertwillow	15	23	6	52	48	8	48
	20	145	39	39	61	51	19
	25	328	88	49	51	87	6
	29	371	100	94	6	78	21

[a] Based on 6-crock replications, each sown with 100 seeds of each species at 4 temperatures. Moderate soil moisture. Viability of seed used: American elm, 43%; Siberian elm, 56%; black locust, 53%; desertwillow, 62%.
[b] Based on sand checks.
[c] Determined from actual stand loss.

Heit and Eliason (4) found that weak seedlings produced by coniferous seed of low viability were unable to emerge through sand. In germination-oven tests, 3 of the species gave slightly higher germination percent-

TABLE 4.—*Summary of groups of microorganisms isolated from 4 broadleaf tree species affected by postemergence damping-off in soil temperature tests*

Soil temperature	Identity of isolate	Total isolations	No. of isolations by tree species			
			American elm	Siberian elm	Black locust	Desertwillow
15° C	Pythium	29	1	8	19	1
	Rhizoctonia	16	4	5	7	0
	Mucoraceae	5	0	1	4	0
	Fusarium spp.	7	0	6	1	0
	Bacteria	6	0	5	1	0
	Miscellaneous	16	1	11	4	0
	Total	79	6	36	36	1
20° C	Pythium	27	4	3	20	0
	Rhizoctonia	27	10	4	9	4
	Mucoraceae	10	1	1	6	2
	Fusarium spp.	6	0	0	5	1
	Bacteria	10	0	1	8	1
	Miscellaneous	26	5	5	14	2
	Total	106	20	14	62	10
25° C	Pythium	50	5	7	32	6
	Rhizoctonia	90	14	12	18	46
	Mucoraceae	17	2	0	14	1
	Fusarium spp.	32	4	6	14	8
	Bacteria	37	4	12	1	20
	Miscellaneous	68	9	15	17	27
	Total	294	38	52	96	108
29° C	Pythium	40	3	2	14	21
	Rhizoctonia	149	1	6	6	136
	Mucoraceae	33	3	5	23	2
	Fusarium spp.	34	5	11	8	10
	Bacteria	33	1	13	1	18
	Miscellaneous	69	8	14	15	32
	Total	358	21	51	67	219
All temperatures		837	85	153	261	338

ages than in sand at similar temperatures. The seed of American elm germinated less than in sand, probably because of partial drying. It appeared, therefore, that seed germination in steam-sterilized sand was sufficiently reliable for determining pre-emergence losses in unsterilized soil.

Soil temperature and infection.—The isolates from each host at each temperature are listed by groups in Table 4. The isolates under "Miscellaneous" were mostly Aspergillus.

In these tests, the effect of temperature on pathogenicity varied with the host species. Black locust proved to be more susceptible to infection by Pythium than to Rhizoctonia at all temperatures, whereas desertwillow was infected more frequently by Rhizoctonia than by Pythium. American and Siberian elm showed a peculiar relationship in this respect. Of the 2 fungi, Rhizoctonia was more commonly isolated from American elm at the 3 lower temperatures than at 29°, but Pythium appeared to dominate at the highest soil temperature. Siberian elm, however, showed the opposite trend, with Pythium predominating at the lowest temperature and Rhizoctonia at the 3 higher temperatures.

On the basis of these data it appears that black locust and desertwillow are good indicator hosts for Pythium and Rhizoctonia, respectively. These hosts should be especially useful in field tests to determine the effect of soil temperature in tests on control of damping-off. A direct comparison is shown graphically in Figure 3.

Temperature and growth of pathogens.—All isolates of *R. solani* and of *P. ultimum* made best growth in malt agar (pH 5.5) at 27° C. *P. ultimum* grew faster than did *R. solani* except at 33° C. There was no constant relationship shown in the rate of growth of the pathogens *in vitro* and postemergence damping-off at similar temperatures. These results agree with those of others that the temperature at which pathogenic fungi grow fastest *in vitro* does not necessarily coincide with that of greatest virulence (6).

Temperature and germination.—The influence of temperature on seed germination and on subsequent growth of the seedlings is of primary importance in damping-off studies. The effect of low temperature on germination was tested by transferring crocks from low-temperature cases to greenhouse temperatures and from high-temperature chambers to the lowest temperature used (Table 5). Low soil temperature was largely responsible for the poor seedling emergence of American elm and desertwillow, whereas for Siberian elm pre-emergence damping-off appeared to be chiefly responsible for reduced germination. Emergence of black locust was not influenced much by low temperature.

Similar but less striking results were obtained when the crocks were transferred from high (33°) to more moderate temperatures (22°–27° C). In sterilized sand, this change was followed by increases in percentage germination as follows: American elm, 11; Siberian elm, 4; black locust, 9; and desertwillow, 0.

Temperature and growth of hosts.—All species except American elm showed a decidedly better growth at 33° than at 16° C at the end of 3 weeks (Table 6). The better growth at 33° was partly due to earlier emergence and was, therefore, not entirely traceable

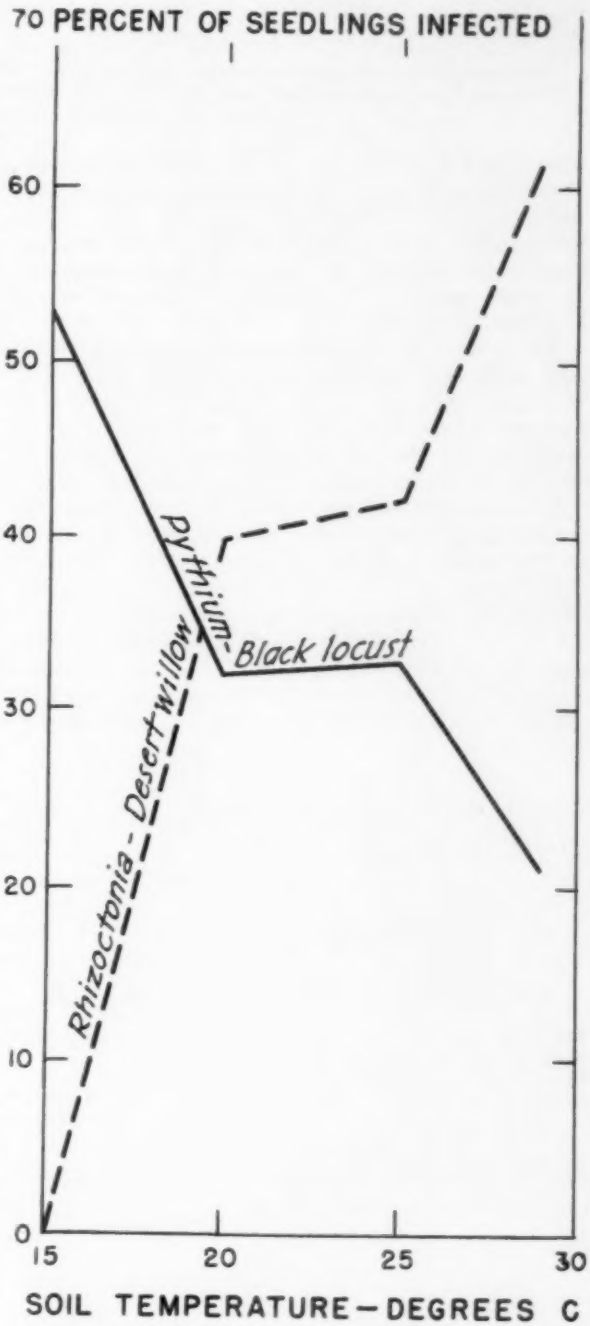

FIG. 3. Graphic presentation of the effect of soil temperature on infection of black locust by Pythium and desertwillow by Rhizoctonia as based on isolations from infected seedlings.

TABLE 5.—*Germination of seed of 4 broadleaf tree species after 5 weeks at air temperature of 16° followed by 1 week at 22°–27° C*

Species	Germination in sterilized sand [a]			Germination in unsterilized soil [b]		
	After 5 weeks at 16°	After 1 additional week at 22°–27°	Increase	After 5 weeks at 16°	After 1 additional week at 22°–27°	Increase
	No.	No.	%	No.	No.	%
American elm	41	88	114	12	22	83
Siberian elm	163	183	12	30	30	0
Black locust	131	147	12	70	78	11
Desertwillow	23	85	270	11	108	882

[a] Two-crock test, with 100 seed sown in each crock.
[b] Four-crock test, with 100 seed sown in each crock.

to increased seedling vigor. At 33°, the height growth of American elm was only slightly better than at 16°, and postemergence damping-off was the same at both temperatures. This is additional evidence that seedling vigor, as influenced by air and soil temperatures, is an important factor in resistance to infection of certain broadleaf tree species.

DISCUSSION.—The indications are that in the nursery, seed drills and seedbeds should be kept moderately wet to promote good germination and growth of broadleaf tree species. Excessive water probably should be avoided at the time of seedling emergence and for a short time thereafter to lessen losses from damping-off.

It is desirable to sow the seed of the 4 broadleaf tree species tested when temperatures are most favorable for germination. Siberian elm and black locust seed can be sown relatively early in the spring, since germination is not seriously retarded by soil temperatures as low as 15° C. American elm and desertwillow seed do not germinate satisfactorily at this low temperature and should be sown later in the spring. American elm seed cannot be sown as late as that of desertwillow, however, since the development of this elm is detrimentally affected by high soil temperatures.

The moderate use of irrigation water and attention to date of sowing undoubtedly will result in reduced damping-off losses. The best time of sowing varies according to locality; in general, sowings should be completed in the southern Great Plains by early April and in the extreme north by the middle of May.

TABLE 6.—*Height growth of 4 broadleaf seedling tree species grown in sterilized sand for 3 weeks at 2 different air temperatures*[a]

Species	3 Weeks at 16°C		3 Weeks at 33°C		Percentage height stimulation
	No. of seedlings	Average height (mm)	No. of seedlings	Average height (mm)	
American elm	42	45	94	50	10
Siberian elm	162	65	150	90	40
Black locust	136	70	104	130	89
Desertwillow	6	10	132	110	91

[a] Two-crock replications used for each species at each temperature.

PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION
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BARN SPOT DISEASE OF FLUE-CURED TOBACCO IN SOUTHERN RHODESIA [1]

R. C. Stephen

SUMMARY

Experiments carried out in temperature-humidity cabinets demonstrated that increases in the yellowing temperature from 85.1° to 94°F had little effect on the development of barn spot disease of flue-cured tobacco leaves during curing caused by *Cercospora nicotianae* Ell. & Ev. At temperatures above 98°F, there was a progressive reduction in the number of barn spots that appeared; however some leaf damage occurred at temperatures above 102°F.

Slight inhibition in development of barn spots resulted when the relative humidity was increased from 85 to 100 per cent when a high yellowing temperature was used.

The fact that barn spot did not develop appreciably when the temperature was 98°F and the relative humidity was 93 per cent indicates that *C. nicotianae*, which does not grow in artificial culture at 93° or above, will not grow in tobacco leaf when conditions are such that the temperature recorded by a wet-bulb thermometer is 96°F or above.

By the use of high yellowing temperatures of 100°–102°F and a relative humidity of 100 per cent, barn spot development has been largely eliminated. Delay in attaining these conditions, even for 8 hours, resulted in appreciable development of barn spot.

More barn spots developed on leaves that were over-ripe when harvested than on leaves that were ripe. The greater the number of original field lesions at harvest, the greater was the number of barn spot lesions that developed during curing. The number of barn spots that developed appeared to be an exponential function of the original incidence and independent of the colouring temperature employed during curing.

The fungus *Cercospora nicotianae* Ell. & Ev. is responsible for frog-eye and barn spot disease of tobacco. The name "frog-eye" is generally applied to those lesions that develop in the field prior to reaping (Fig. 1), whereas barn spot develops during curing (Fig. 2). The extension of lesions already present and the development of new spots in the barn during curing, referred to as barn spots, are the more economically important phases of the disease that has been widespread throughout Rhodesia. Financial losses due to the prevalence of heavily barn-spotted leaf were known to be considerable, and experiments were carried out to determine the influence of factors associated with flue-curing on barn-spot development, with a view to introducing control measures.

REVIEW OF LITERATURE.—Although workers (3, 4, 6) agree that delay in reaping results in a substantial increase in the amount of field frog-eye, the effect of leaf maturity at reaping on the subsequent development of barn spot does not appear to have been examined previously. Mandelson (6) noted that the more mature the leaf tissue the more liable it was to barn-spot development, but his experiments were concerned with the extension of field spots and did not deal specifically with the new lesions that appeared during curing.

Reports regarding the effects of temperature and humidity variation on barn-spot incidence and the control measures suggested by various workers differed considerably. Butler, cited by Hill (3), recorded that spot extension during curing could be checked by raising the temperature for a time to 120°F or more. A similar method reported by Hopkins (4) consisted of increasing the barn temperature to 160°F immediately after filling the barn; once this temperature had been attained, the barn was allowed to cool and thereafter the normal curing procedure was adopted. Hopkins noted, however, that there was a certain amount of risk owing to the danger of ruining leaf colour. Mandelson (6) concluded that the danger of overheating was too great to warrant general recommendation of these methods, although they did control barn spotting to some extent.

Other control measures were suggested by Jochems, cited by Hill (3), who reported that barn spotting was partially controlled by ample ventilation, and by Hopkins (4), who recommended top ventilation and low moisture during the early stages of curing.

In preliminary studies in commercial curing barns, Mandelson (6) demonstrated that barn spotting was less when relative humidities of 96 per cent or more were maintained during colouring than when the relative humidity was 90 per cent; he concluded tentatively that humidity rather than high temperatures (98°–108° compared with 82°–100°F) must be responsible for the control. Hill (3), however, concluded that variations in temperature and humidity had no significant influence on barn-spot incidence. In his experiments, colouring temperatures from approximately 77° to 96.8°F were tested in association with low and high relative humidities.

Hopkins (5) further observed that very few barn spots appeared if curing was commenced at 100°F with 100 per cent relative humidity "even though the atmosphere is saturated as it must be in order to prevent scorching and allow colouring to proceed." Carr (2), however, reported less barn spot than usual when colouring was carried out at lower temperatures

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Research Paper No. 31, Tobacco Research Board of Rhodesia and Nyasaland, Salisbury, Rhodesia.

[2] Senior Biologist, Levington Research Station, Ipswich, Suffolk, formerly of Tobacco Research Board of Rhodesia and Nyasaland.

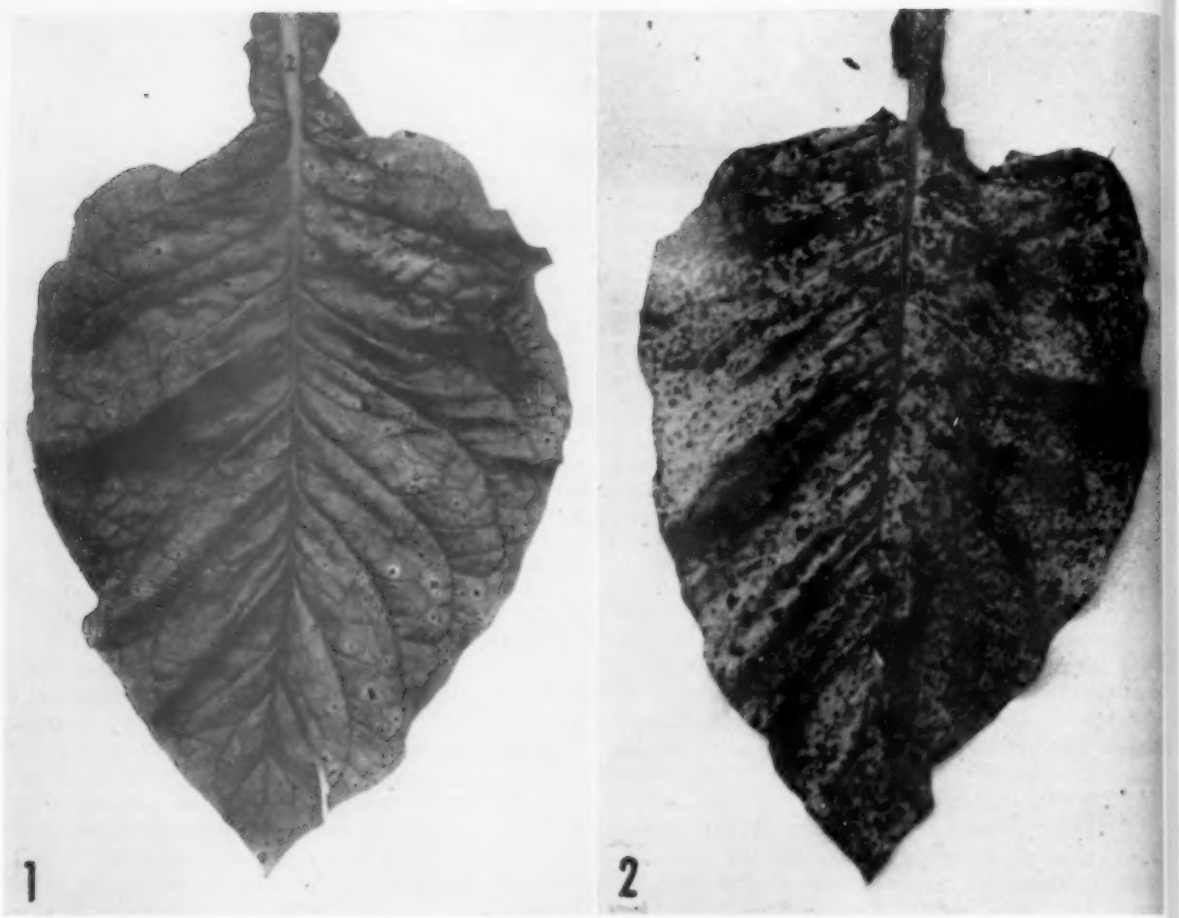

FIG. 1–2. Fig. 1. Ripe tobacco leaf before curing, showing frog-eye as it occurs in the field.—Fig. 2. Same leaf as shown in Figure 1 after it had been cured under conditions favourable for development of barn spot.

than usual but with fairly rapid drying out of web and midrib.

MATERIALS AND METHODS.—Leaves of uniform ripeness, from approximately the same position on the plant, were selected for test, except when the factors of leaf spotting and/or leaf maturity at reaping time were under study; in such cases, leaves were selected for these differences in the field.

Lesion counts were made within sample areas, consisting of two 2-in. squares in approximately similar positions on each half leaf. The number of barn spots was determined by recording the numbers of spots within the faintly marked squares before and after colouring. The numbers of barn spots were calculated on the basis of an average-sized leaf; this was taken as a leaf with a 2-ft. midrib and 1 ft. in width at the widest point. The area of such a leaf is approximately 200 sq. in.

Leaves were split longitudinally along the line of the midrib and the halves distributed randomly among treatments. The half-leaves were suspended from ½-in. flat metal "needles" passed through the midrib at the butt end. Trials were carried out in cabinets in which dry and wet-bulb temperatures were controlled within ±0.5°C.

The end point of the experiments was defined as that stage at which the leaves had a uniform yellow appearance except for a green shade or tinge in the veins. Except for those tests concerned with leaf maturity, the colouring period for different treatments was approximately the same; with leaves differing in maturity, yellowing was continued until the appearance of the leaf had reached the point defined above.

EXPERIMENTAL WORK.—*Influence of colouring temperature.*—As a result of preliminary experiments designed to test the effect of as wide a temperature range as practical, the temperature range tested was narrowed to determine which one showed the least development of barn spot without detrimental effect to the leaf.

Ripe leaves exhibiting only a few original lesions were used throughout. The relative humidity was kept constant at 93 per cent in all tests.

Increasing the colouring temperature from 85.1° to 94°–98°F had little or no effect on barn-spot develop-

ment (Table 1). Above 100°, markedly fewer barn spots developed than at lower temperatures. The most favourable temperature for large reductions in barn spot development appeared to be 102°F; furthermore, this colouring temperature had no adverse effect on the leaf.

Damage, in the form of "scorching" and incomplete yellowing, resulted from the 105.8° treatment. Consequently, this high yellowing temperature was considered impractical, despite the very low number of barn spots recorded.

Influence of relative humidity.—A correctly packed barn should be capable of providing sufficient moisture from the leaves to permit colouring to proceed at low initial temperatures of 85°–95°F. At higher temperatures of 100°–102°, however, a high humidity is required because of the danger of drying of the leaf before the correct colour has been obtained. The effect of variation in relative humidity on barn spot development was studied in several trials.

Relative humidities of 85 and 100 per cent at yellowing temperatures of 90° and 100°F, were tested on ripe leaf showing a low original incidence of spotting.

Increase in relative humidity from 85 to 100 per cent apparently decreased the amount of barn spot when the yellowing temperature was 100° but not when it was 90°.

Influence of leaf maturity and original incidence of field frog-eye at time of reaping.—The factors of leaf maturity and the number of frog-eye lesions present at reaping were studied together, since it seemed that the incidence of field spot depended on the age of the leaf. It is known (3, 4, 6, 7) that if leaf is allowed to become over-ripe before reaping there is a substantial increase in the number of visible frog-eye lesions. For this reason it proved impossible to select ripe and over-ripe leaf bearing approximately the same number of field spots. Over-ripe leaf selected in the field showed less than 3 times the number of frog-eye lesions showed by the ripe leaf. In contrast, the numbers of field lesions present on leaf classified in the high-original-incidence category were at least 9 times more numerous as those on leaves in the low-original-incidence category.

The effects on barn-spot development during yellowing of ripe and over-ripe leaf with a low and a high original incidence of field spots were examined separately and together in a number of trials. The more mature the leaf at reaping and the greater the incidence of spotting on the leaf at this time, the greater was the number of barn spots which developed during yellowing (Tables 2 and 3).

In addition, these trials (Tables 2 and 3) demonstrated that with the use of higher colouring temperatures of 100° and 102°F fewer barn spots developed than with temperatures of 90° and 92° irrespective of the leaf maturity and of the original amount of field spotting at harvest. These results confirmed

TABLE 1.—*Barn-spot development on tobacco leaves at various yellowing temperatures*

Yellowing temperature (°F)	No. of barn spots/200 sq. in. leaf		
	Trials 1–3	Trials 4–5	Trial 6
85.1	518		
90		330	
92.3	440		
94		298	
98		228	294
98.6	163		
100			175
102		28	58
105.8	48		
Standard error	(±47.4)	(±28.9)	(±32.3)
LSD (0.05)	135	82	93
(0.01)	180	108	125

TABLE 2.—*Barn-spot development during colouring of tobacco leaves that differed in maturity and incidence of spotting at time of reaping in 3 trials at a relative humidity of 93 per cent*

Leaf maturity	Original incidence of spotting	No. of barn spots/200 sq. in leaf at indicated temperature (°F) [a]		
		92°	102°	Mean
Ripe	Low + high	471	102	286
Over-ripe	Low + high	431	124	278
Ripe +	Low	293	76	184
Over-ripe	High	609	150	380
Mean		451	113	282

[a] Standard errors and least significant differences for comparison of means were as follows: for main effects—standard error = ±23.6, LSD = 67 (P = 0.05) and 88 (P = 0.01); for 2-factor interactions—standard error = ±33.4, LSD = 94 (P = 0.05) and 124 (P = 0.01).

TABLE 3.—*Barn-spot development during colouring of tobacco leaves that differed in maturity and incidence of spotting at time of reaping in 2 trials at a relative humidity of 100 per cent*

Leaf maturity	Original incidence of spotting	No. of barn spots/200 sq. in. leaf at indicated temperature (°F) [a]		
		90°	100°	Mean
Ripe	Low	373	253	313
	High	1267	738	1002
Over-ripe	Low	563	320	442
	High	2025	1234	1630
Ripe	Low + high	820	495	658
Over-ripe	Low + high	1294	777	1036
Ripe +	Low	468	286	377
Over-ripe	High	1646	986	1316
Mean		1057	636	847

[a] Standard errors and least significant differences for comparison of means were as follows: for main effects—standard error = ±46.3, LSD = 131 (P = 0.05) and 173 (P = 0.01); for 2-factor interactions—standard error = ±65.5, LSD = 185 (P = 0.05) and 244 (P = 0.01); for 3-factor interaction—standard error = ±92.6, LSD = 261 (P = 0.05) and 345 (P = 0.01).

those tests (Table 1) involving similar temperatures but which employed only ripe leaf with a low initial incidence of field spotting.

A very highly significant correlation ($P<0.001$) was found between the original and final incidence, a relationship that appeared to be related to temperature. The difference between the number of barn spots that developed on leaves that had a high initial incidence of frog-eye and the number on leaves that had a low initial incidence was greater at the lower curing temperature than at the higher. At both temperatures, however, the proportionate increase in numbers of barn spots, on leaves with a low and high initial incidence, was almost identical. This was shown clearly when the logarithms of the number of barn spots at the 2 temperatures were plotted against the number of initial field spots (Fig. 3).

It appears that the incidence of barn spot is an exponential function of the incidence of field spot and that the shape of the curve representing this function is independent of temperature. It should be emphasized, however, that an insufficient number of classes of original incidence was available to permit a precise definition of this relationship.

Practical application of results.—Experiments detailed up to this point have been concerned only with the separate effects of various factors, or of pairs of factors, on the amount of spotting that developed during curing. From the practical point of view, the main interest is in the total amount of spotting (i.e. initial field spotting plus barn spots) on the cured leaf. The total number of lesions present after yellowing was examined in 2 trials in which all possible combinations were made of the following variables: temperatures of 90° and 100°F, relative humidities of 85 and 100 per cent, leaf that was ripe and over-ripe, and a low and high original incidence of field frog-eye.

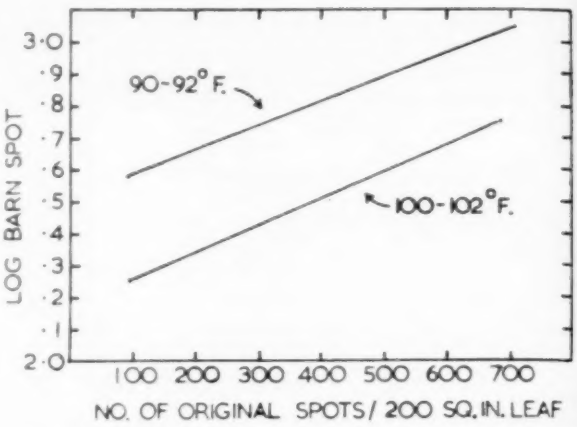

FIG. 3. Logarithms of number of barn spots developed at 2 different temperatures as a function of the number of field lesions (frog-eye).

Leaf with a low original incidence, harvested when ripe and yellowed at a temperature of 100°F with a relative humidity of 100 per cent, had the least total amount of spotting following yellowing (Table 4). Leaf with a high original incidence, harvested when over-ripe and yellowed at a temperature of 90°F with a relative humidity of 85 per cent, had the greatest total amount. It would appear that the yellowing temperature and the original incidence at harvest are the major factors and that incidence of barn spot will be low if the field incidence can be kept at a low level and a high temperature (100°–102°F) is used during the yellowing phase of curing. At this high temperature, the use of humidities approaching saturation will also effect a further improvement in control.

Further experiments were carried out to determine whether delay in the attaining of these temperatures

TABLE 4.—*Total number of lesions (original field frog-eye plus barn spots) when tobacco leaf of different maturity and incidence of frog-eye when harvested was cured at different colouring temperatures and relative humidities in 2 trials*

Condition of leaf when harvested		No. of lesions/200 sq. in. leaf when coloured at indicated temperature and relative humidity[a]				
		90°F		100°F		
Maturity	Incidence of frog-eye	85%	100%	85%	100%	Mean
Ripe	Low	390	491	388	259	382
	High	1697	1916	1412	1403	1607
Over-ripe	Low	634	728	456	384	551
	High	3003	2959	2209	2012	2546
Ripe	Low + high	1044	1203	900	831	995
Over-ripe	Low + high	1819	1844	1333	1198	1548
Ripe + over-ripe	Low	512	609	422	322	466
	High	2350	2438	1811	1708	2077
Mean		1431	1523	1116	1015	1271

[a] Standard errors and least significant differences for comparison of means were as follows: for main effects—standard error = ±52.4, LSD = 148 (P = 0.05) and 195 (P = 0.01); for 2-factor interactions—standard error = ±74.0, LSD = 209 (P = 0.05) and 276 (P = 0.01); for 3-factor interactions—standard error = ±104.7, LSD = 295 (P = 0.05) and 390 (P = 0.01); for 4-factor interactions—standard error = ±148.1, LSD = 417 (P = 0.05) and 552 (P = 0.01).

had any effect on the incidence of barn spot. In the first trial, the temperature in 2 treatments was maintained at 90°F for 8 and 16 hours before being raised to 100°F. In the third treatment, a temperature of 100° was used from the beginning.

When yellowing was carried out at a temperature of 100° from the beginning, there was considerably less barn spot development than when this temperature was attained after a delay of either 8 or 16 hours; an average of 98, 258, and 363 spots per 200 sq. in. leaf developed, respectively. The data suggest that the latent infections became visible between 8 and 16 hours after curing had started. This period would vary according to leaf type and age of leaf at harvest.

In a further trial, the general practice of stepwise increases in the curing temperature until 100°F is reached, as recommended by Brown (1), was compared with curing at a continuous temperature of 100°. Curing at 100° for the entire period, at either 85 or 100 per cent relative humidity, resulted in much less barn spot than did a curing schedule in which the temperature was raised to 100° in 4 steps over a 32-hour period with the relative humidity maintained at 92 per cent (Table 5). When the latter schedule was used, some lesions became visible as early as 8 hours after the cure was started. When an 8-hour period at 90° preceded attainment of a temperature of 100°, incidence of barn spot was less than when the leaf was cured at 100° throughout; however the difference in incidence following the 2 treatments was not quite statistically significant (Table 5).

Application of results in commercial curing barns.—The information gained in the foregoing trials has been applied under farm conditions. Humidities approaching saturation were attained either by use of an electric humidifier or by the passing in of steam at a boiler pressure of 25–30 lb. sq. in. Temperatures of 100°–102°F were attained by using the furnace in the case of the electric humidifier, but this was unnecessary when steam was used since the steam itself supplied the required heat. The use of steam appears to be the more practical of the 2 methods because it is easier to manage; in addition, more growers are equipped for steam than for electric humidifiers.

The technique employed when steam was used consisted of leading it into a U-shaped unit of ¾-in. piping punched with ⅛-in. holes 3 in. apart in a single line to facilitate even distribution throughout the barn. Initially, the steam was passed rapidly into the barn to attain temperatures of 100°–102°F, together with a relative humidity of 100 per cent, as soon as possible. Thereafter, the supply was reduced to a level sufficient to maintain these conditions. In both methods, the supplying of moisture was stopped after 20 hours and the humidifier or steam tube removed, the furnace meanwhile being adjusted to keep the temperature at 100°–102° without an intervening drop.

The steam method has been tested both at experimental stations and on commercial farms; its use, when barn spot was prevalent, controlled the disease. If use of the technique was discontinued in a season when barn spot was a problem, the disease again became prominent on the cured leaf but was checked on reapplication of the technique involving 100°–102°F yellowing temperature and a relative humidity of 100 per cent.

TABLE 5.—*Barn spot development on tobacco leaf when the colouring temperature during curing was 100°F throughout and when it was raised to 100° stepwise*

Temperature (°F) during consecutive 8-hour periods and the final temperature maintained until colouring was completed					Relative humidity (%)	No. of spots 200 sq. in. leaf*
1	2	3	4	Final		
85	90	95	95	100	92	1239
90	100	100	100	100	92	990
100	100	100	100	100	85	783
100	100	100	100	100	100	655
Standard error						±115.2
LSD (P = 0.05)						350
LSD (P = 0.01)						485

* Adjusted for differences in original incidence by analysis of covariance.

DISCUSSION.—The direct effect of high air temperatures and humidities on development of *C. nicotianae* within host tissues is unknown; however, it is suggested that the observation that *C. nicotianae* does not grow on artificial media above a temperature of 93°F (6) has a bearing on the finding that high temperatures inhibit the development of barn spot. The failure in the past to recognize this possible relation might have been due to attempts to compare directly the temperature within the leaf tissues with the air temperatures (recorded by a dry-bulb thermometer) existing in the barn during the yellowing period of curing. The results of the present work indicate that the critical temperature for barn spot development is 98°F at a relative humidity of 93 per cent; these conditions correspond to a wet-bulb thermometer reading of 96°, which presumably would approximate the temperature within a tobacco leaf during the yellowing phase of curing. It would seem that under the conditions of the tests, the maximum temperature at which growth of the fungus can take place within the host is about 96°F. Since conditions within the leaf may be more favourable for growth of the fungus than are those provided by artificial media, it is possible that the upper limit for growth might differ under the 2 conditions.

The conclusions regarding the temperature effects do not appear to agree with those of Mandelson (6) and Hill (3), who reported that temperature had no effect on barn spot incidence. Mandelson was unable to reconcile the fact that barn spotting occurred when the temperature during curing did not fall below 95°F, at which level the fungus did not grow on artificial media. The control he obtained was tentatively

attributed to the maintenance of relative humidities of 96 per cent or more; however, in association with these high humidities Mandelson employed temperatures varying between 98° and 108°F. In view of the present evidence, the control must have been due primarily to the high temperatures.

In the case of Hill's (3) work, the highest temperature tested was approximately 96.8°F; in fact, this temperature was only attained about 16 hours after the curing began. Since the relative humidity was below 100 per cent for most of the trial, the wet-bulb temperature must have been below the critical level postulated by the writer; accordingly the failure to control barn spot can be understood.

Hopkins' (5) suggestion that barn spotting could be prevented by beginning the cure at a temperature of 100°F was only partially confirmed, since the use of such a temperature during yellowing is not sufficient in itself to control barn spot. Earlier, Hopkins (4) recommended low-moisture conditions during the early stages of curing. Later, he implied (5) that the use of a high humidity in association with a temperature of 100°F was not conducive to barn spot control but that it was a necessary concomitant to prevent leaf damage. From the writer's results, it would seem that a relative humidity of at least 90 per cent is necessary for barn spot control and for prevention of leaf damage when a curing temperature of 100° is used.

Other trials demonstrated that the high temperatures and humidities must be attained as soon as possible after curing is commenced, since a delay of even 8 hours caused an increase in barn spot development. Apparently, the interval between 8 and 16 hours after curing commenced was the critical time for development, an observation in line with Mandelson's (6) observation that barn spot appeared approximately 12 hours after curing began. The time at which barn spots become visible will vary, however, according to various factors, including the speed at which yellowing takes place, the position on the plant from which the leaf is taken, and the age at time of harvest.

The data on the influence of leaf maturity on barn spot development confirmed Mandelson's (6) observation that the more mature the leaf tissue, the more liable it is to the development of barn spots.

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BARK CANKER, A BACTERIAL DISEASE OF THE PERSIAN WALNUT TREE [1]

E. E. Wilson, Mortimer P. Starr, and Joyce A. Berger

SUMMARY

An apparently undescribed disease of Persian (English) walnut was found in the Sacramento Valley of California in 1955. The disease is characterized by extensive, shallow, irregularly shaped cankers in the bark of the trunk and scaffold branches of mature trees. Cankers expand throughout the summer but are inactive in winter. A bacterium that is present in large numbers in the affected bark proved to be the causal organism. It is a gram-negative, peritrichous, nonpectinolytic bacillus related to the fireblight organism, *Erwinia amylovora*. By direct comparison, it was possible to distinguish the 2 organisms with sufficient certainty to warrant the designation of the walnut pathogen as *Erwinia nigrifluens* sp. nov.

In 1955 a disease was found in a Persian (English) walnut orchard in Glenn County, California. Later it was found in Sutter, Yolo, and Colusa counties. All of the counties are located in the Sacramento Valley. Examination of orchards in Santa Clara, Santa Barbara, Napa, Sonoma, and Tulare counties indicate that the disease is not prevalent in other parts of the state. So far as we are aware, this is a disease that has not been described before.

SYMPTOMS.—The disease, which has been given the name "bark canker," is characterized by the formation of irregular dark brown necrotic areas in the bark of the trunk and scaffold branches (Fig. 1). The necrotic areas are at first small circular spots in the cortical tissue just beneath the corky outer layer of bark (phelloderm). Extensive, irregularly shaped cankers are formed by the enlargement and coalescence of several such areas.

The outline of the canker is not visible from the surface, but its presence usually can be detected by a dark-colored watery material that escapes through small cracks and stains the surface of the bark (Fig. 2). At times, the dark sap accumulates in a pocket formed by a radial break in the tissue beneath the phelloderm. When the phelloderm is punctured with a knife, the sap squirts out with considerable force.

In general, the canker is relatively shallow, extending only about one-fourth to one-third the depth of the bark. When, however, a radial break develops as described above, the bark tissue adjacent to the crack becomes involved to a greater depth. Under such circumstances, the phloem adjacent to the cambium may be killed. Usually, however, extensive necrosis occurs only in the outer or cortical tissue.

In some features, bark canker resembles melaxuma (2), a disease caused by the fungus *Dothiorella gregaria* Sacc. Both are characterized by large bark cankers that exude a dark watery material. The cankers of melaxuma occur most commonly at the crotches between large limbs, whereas cankers of bark canker occur on the trunk in no particular relation to crotches. In melaxuma cankers, the bark down to the wood is killed, and there is some discoloration of the underlying wood; in contrast, bark cankers seldom extend down to the wood. The similarities between the 2 diseases, however, are more readily perceived than

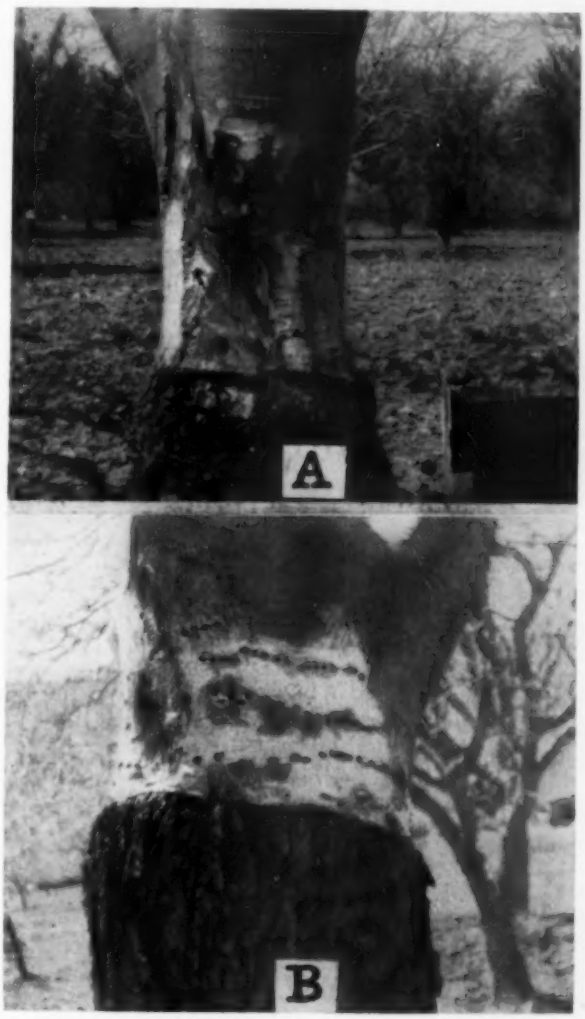

FIG. 1. A) Phelloderm removed from the trunk of an affected tree, exposing the extensive irregularly shaped canker below. B) Infection adjacent to the holes produced by sapsuckers in the trunk of a walnut tree.

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We are obliged to Dr. Donald D. Sutton for assistance in performing and interpreting some critical determinative tests and for stimulating discussions concerning the taxonomic position of the walnut canker pathogen.

FIG. 2. The trunk of a Persian walnut tree affected by bark canker. Black sap has exuded from the canker and dried on the surface of the bark. The tree is growing on black walnut (*Juglans hindsii* Jepson) understock.

are the dissimilarities. It is not unlikely, therefore, that in the past, bark canker has been confused with melaxuma.

Since bark canker has been recognized as a distinct disease for only a year, we cannot predict exactly its effect on the tree. Judging from the extensive nature of the cankers, one might expect it to be capable of causing serious damage. We know of trees that have had the disease possibly for 3 or 4 years. Exfoliation of the affected portions of bark has occurred over large areas of the trunks of these trees, but neither the vigor nor productivity of the trees has been noticeably affected so far. Apparently any effect of the disease will be slow to develop.

So far, no case of bark canker has been found on trees less than about 10 or 15 years old, and most of the affected trees are older than that. Moreover, the cankers occur on the trunk and scaffold branches, which of course are some of the oldest parts of the tree. No cankers of a similar nature have been found elsewhere on the tree.

SUSCEPTIBILITY OF WALNUT VARIETIES.—Bark canker has been found in trees of the following varieties: Hartley, Mayette, Payne, Mammoth, Meylan, and Myrtleford. The first 3 are important varieties in California, whereas the others are of relatively little importance. Franquette, the most widely planted variety in the walnut-growing areas north of the Tehachapi Mountains, is relatively free of the disease. For example, in an orchard where at least 75 per cent of the Hartley trees are affected by bark canker, none of the Franquette trees are affected.

RESULTS OF ISOLATION FROM AFFECTED BARK.—Bacteria were isolated repeatedly from the affected bark in the following manner. Small pieces of bark were removed under aseptic conditions and placed in separate 10-ml lots of sterile water. After ½ hour or more, 1 or 2 drops of the water were suspended in melted potato-glucose agar and the agar poured into petri plates. The water often contained such large numbers of bacteria that it was necessary to dilute it in order to obtain separate colonies. A number of isolates of these bacteria were used in subsequent inoculation and cultural studies. The distinctive growth of the bacterium on EMB agar (containing eosin, methylene blue, lactose, and sucrose, as described in a later section) proved valuable as a means for tentatively identifying the bacterium in these and other isolation studies.

Pieces of the bark also were placed on the surface of hardened potato agar. Only in a few cases did fungi grow from these pieces, and these proved to be common saprophytes such as species of *Penicillium* and *Alternaria*.

INOCULATION EXPERIMENTS.—On June 24 and on July 8, 1955, 2 isolates of the bacteria obtained from the typical bark cankers were inoculated into the trunk and scaffold branches of 6-year-old trees of the Franquette variety. The surface of the bark was first wiped with 95 per cent ethyl alcohol. Next a fine needle was inserted tangentially into the bark, the bacteria in water suspension were injected into the hole with a hypodermic needle, and the openings were sealed with adhesive tape.

Four weeks after inoculation, the bark tissue above and below the needle punctures had been killed for a distance of about ¼ in. Black sap had exuded from the lesions at the site of most inoculations. On October 3, 1955, bacteria that resembled those used in inoculation were recovered from the lesions.

On October 13, 1955, other Franquette trees in the same orchard were inoculated in a similar manner. During the autumn, lesions about ¼ in. long developed around the path of the needle. These showed no signs of progress during the winter, but in spring they began to expand slowly and some attained a length of ½ in. by summer. Bacteria were readily isolated from these lesions. When grown on the special differential EMB medium, the bacteria were found to resemble those used in the inoculations. The bacterium was isolated from affected trees at all seasons

of the year. It was found in large numbers in the sap that exudes from the cankers during the growing season. It remained alive in the dried residue of the sap for at least a week.

After observations in the orchard had indicated that Franquette is seldom attacked by bark canker, other varieties were used for inoculation tests. The orchard used for these tests contained most of the principal horticultural varieties grown in the state, including reportedly slightly different clones of some of these varieties. For example, in addition to the original Franquette variety, trees of the Andross, Simonet, and Vrooman Payne clones of Franquette were inoculated. The test also included the varieties Hartley, Mayette, Waterloo, Woodland, and Poe.

Inoculations in this orchard were made February 29 and March 30, 1956. By May, the bacteria had killed small areas of the bark adjacent to the needle punctures in all inoculated trees. In trees of the Vrooman Payne Franquette and Simonet Franquette, however, the lesions were much larger and exhibited signs of greater activity than the lesions in trees of the Andross Franquette. By August, cankers 3–4 in. long had developed around some of the needle punctures in trees of the Vrooman Payne and Simonet clones. In contrast, lesions from 5 to 8 in. long had developed around most of the needle punctures on Hartley and Mayette. Bacteria similar to those used in inoculation were recovered from the cankers. Inoculated trees of the Waterloo, Woodland, and Poe varieties did not develop the disease.

The bacteria were introduced into the stems of young squash, bean, pea, and tomato plants and into the leaf petioles of young sugar beet plants. The inoculated plants were incubated in a moist chamber for 48 hours at an average temperature of 75°F and were then placed on the greenhouse bench. No disease symptoms developed.

The bacteria failed to produce symptoms in the growing shoots of pear seedlings and in green pear fruit. Under the same conditions, *Erwinia amylovora* (Burr.) Bergey et al produced extensive symptoms in 4 or 5 days.

When the bacterium was introduced into the bark of the branches of almond trees (Nonpareil variety) on April 14, 1956, lesions 1–3 in. long were produced within 2 weeks. Although the lesions expanded rapidly during early May and the bacteria were easily recovered at that time, they became inactive in late May and did not thereafter resume activity.

STUDIES ON THE WALNUT BARK CANKER PATHOGEN.—*Methods and procedures in cultural studies.*—Twenty-five cultures (10 isolates and 15 reisolates) of the bacterium associated with the walnut bark canker disease were subjected to the determinative procedures described in previous publications (7, 9, 10). Purity of all cultures was assured in the following manner. The original cultures, which formed sparse, glossy, white growth on slants of potato-glucose agar (PDA), were transferred to Albimi Brucella Broth. Within 24 hours, a heavy growth resulted. These cultures were checked for purity by streaking on plates of YDC agar (containing yeast extract, glucose, and calcium carbonate). Colonies were picked at the end of 24 hours, suspended in sterile water, and restreaked. This procedure was repeated until the macroscopic and microscopic appearance indicated that pure cultures had been obtained. Stock cultures were maintained on YDC slants at 4° C. Except where indicated otherwise, determinative tests were carried out at 28° C.

All the pure cultures were used in preliminary tests and behaved similarly. Thereafter, 3 of the original isolates and 1 reisolate, all of which had been used in pathogenicity tests on walnut, were studied in detail, including comparison with authentic cultures of *Erwinia amylovora*.

Morphology and staining reactions.—Living cells, as observed with the phase microscope, are rod-shaped and have slightly pointed ends; many of the cells appear somewhat curved. They occur singly and in short chains. Living cells from a 24-hour-old culture on YDC agar have an average size of 1.1×2.6μ (1.0–1.3×1.7–3.5μ); comparable cells from Albimi Brucella Broth have an average size of 0.8×2.2μ (0.6–1.0×1.6–3.0μ). They contain dark granules scattered throughout the cell, with a somewhat larger granule at each pole.

Gram-stained smears show a gram-negative reaction with some retention of the gentian violet in the granules, particularly at the poles.

Wet mounts prepared from young cultures in broth or agar, or from YDC cultures up to 1 month old, indicated that the walnut pathogen is motile. By means of Leifson's flagella staining procedure (4), peritrichous flagellar arrangement was demonstrated. During the air-drying procedure, most of the flagella were ripped off the cells. This was partly avoided by spraying bacteria onto the slides with a fine atomizer. There are at least 5 flagella per cell, and each flagellum is 2–3 times the length of the cell.

Endospores are not formed, as indicated by direct observations under the phase microscope of old cultures in liquid and agar media.

No capsules were seen by direct observations under the phase microscope, by negative staining with nigrosin, or by staining with the Hiss capsule stain.

Cultural characteristics.—On YDC agar, colonies from a 24-hour culture are white, circular, and raised, with entire margins. On plates prepared with thin layers of YDC agar, colonies are surrounded by a clear zone, which indicates acid production with the dissolution of the calcium carbonate.

When the pathogen was streaked on plates of agar containing eosin, methylene blue, sucrose, and lactose (Bacto EMB agar), dark violet colonies with a green metallic sheen appeared. On EMB agar containing only lactose and no sucrose, however, the colonies were moist and pink. Because of the distinctive growth of the walnut canker bacteria on Bacto EMB lactose-sucrose agar, this medium was used extensively

in the isolation studies described in an earlier section.

Growth occurred on the following agar media: potato-glucose agar (Bacto and freshly prepared); potato-glucose agar plus 0.5 per cent peptone (freshly prepared); Bacto Eugon agar, with and without 2 per cent $CaCO_3$; yeast-extract agar; nutrient agar containing beef extract and peptone; and yeast-extract-glucose agar. No growth occurred on Bacto potato-glucose agar containing peptone or on wort agar. Growth was scanty on nutrient agar containing beef extract and peptone.

In Albimi Brucella Broth, the bacteria produced an abundant growth throughout the tube in 24 hours; growth was heaviest at the surface. In 48 hours, many of the bacteria had settled to the bottom of the tubes, but the medium remained turbid. In undisturbed liquid cultures in nutrient broth, growth was scanty and appeared only at the surface; in cultures that were shaken continuously, however, heavy growth occurred throughout the medium.

Autoclaved litmus milk (Bacto) was gradually turned blue; within a month, the litmus was reduced at the bottom of the tube.

The walnut pathogen is facultatively anaerobic, as shown by its prompt growth at the surface of nutrient-agar shake tubes followed by scantier growth throughout the agar.

No growth occurred on the surface of autoclaved potato cylinders or in the liquid below the potato.

No distinct coloration of the cell mass or of media was detected.

Temperature relations.—Cultures on YDC plates were incubated at 20°, 24°, 28°, and 37° C. In 17 hours, there was no growth at 20°, some growth at 24°, heavy growth at 28°, and no growth at 37°. In 40 hours, growth had appeared at 20°, but none had appeared at 37°. Cultures stored at 4° did not grow detectably, but maintained viability for more than 1 month.

The pH limits for growth.—The walnut canker pathogen was streaked on yeast-extract-glucose agar that had been adjusted to hydrogen-ion concentrations ranging from pH 3.9 to 8.6. Good growth occurred at pH 6.3, 7.8, and 8.6; fair growth developed at pH 5.9, but there was no growth at pH 3.9 or 4.9. Retardation of growth on acid media is fairly common for phytopathogenic bacteria (9), thus pointing up the desirability of using media somewhat closed to neutral reactions than that of the potato-glucose agar commonly used for isolation of phytopathogens from infected plants.

Physiological characteristics.—The walnut pathogen grew moderately well when a washed inoculum was introduced into a synthetic medium containing phosphate buffer, ethylenediamine tetraacetic acid, magnesium sulfate, calcium carbonate, "trace metals," and a mixture of purified amino acids (3, 6). The addition of a sugar (sucrose or glucose), or of a mixture of purified vitamins, increased growth slightly. The pathogen did not grow with glucose as sole carbon source and ammonium chloride as sole nitrogen source, but it did use amino acids as the sole source of carbon or nitrogen.

Two basal media were used for determining action on sugars, with similar results. One was 0.3 per cent beef extract plus 0.5 per cent peptone and bromthymol blue, and the other was 0.5 per cent trypticase broth with bromthymol blue plus chlorphenol red. Durham tubes were employed, and carbon sources were added at the 0.5–1.0 per cent level. Growth and acid production, but no gas, developed in media containing glucose, fructose, galactose, mannose, ribose, xylose, arabinose, rhamnose, sucrose, cellobiose, raffinose, α-methylglucoside, salicin, mannitol, or sorbitol and in media containing glucuronic acid or mucic acid. No acid developed in media containing lactose, dulcitol, adonitol, gluconic acid, or galacturonic acid.

Action on disaccharides was tested in a synthetic basal medium containing phosphate buffer, inorganic salts, an amino acid mixture, and a vitamin mixture. Growth and acid production occurred in the synthetic sucrose medium but not in the lactose or maltose media. A scanty growth in the maltose medium and a rise in pH can probably be ascribed to organic contaminants of this sugar.

When the organism was grown on Bacto MR-VP medium, the methyl red test was positive after 20 hours but negative after 48 hours; the Voges-Proskauer test was negative after 20 hours, slightly positive after a week, and distinctly positive in a month.

On cellulose agar (0.5 per cent Bacto Casamino acids, 0.2 per cent finely shredded filter paper, 1.5 per cent agar), there was good growth but no dissolution of the cellulose.

Good growth, but no blue color, occurred on spirit blue agar containing cottonseed oil (5, 8), an indication that the fat is not simply hydrolyzed. It must be mentioned, however, that such an action as oxidation of fat is not detectable by this indicator method; hence to avoid ambiguity, this negative action of the walnut canker pathogen is best reported as "no blue color on spirit blue fat agar," rather than "not lipolytic."

Hydrogen sulfide production by the pathogen was detectable by means of lead acetate paper suspended over cultures in BBL thiotone broth. No hydrogen sulfide was detected, however, when the bacterium was growing in Bacto triple sugar iron agar.

The pathogen was streaked on starch agar plates containing 0.2 per cent soluble starch and 0.5 per cent yeast extract. After 10 days, a small clear zone was evident around the colonies when plates were flooded with Lugol's iodine solution. Further incubation did not increase the size of the zone. This shows, therefore, that the pathogen is capable of a slow weak hydrolysis of starch.

Sodium ammonium pectate medium, with or without yeast extract (7), was not liquefied. As mentioned before, galacturonic acid is not fermented.

In nutrient-gelatin stabs incubated at 22° and 30°C,

growth was poor and a slow liquefaction was detected with difficulty. When the pathogen streaked on agar plates containing 0.4 per cent gelatin was incubated 10 days and then flooded with $HgCl_2$, however, gelatin digestion was indicated.

Although the pathogen grew rapidly in Bacto tryptone broth, Kovacs' reagent indicated that no indole was produced after 24 hours or after a week.

Suspensions of the pathogen were spread on Bacto beef heart infusion agar and allowed to dry. Bacto Sensitivity Discs containing various antibiotics at several concentrations were placed on the plates. Plates were incubated at 28°C for 48–72 hours or until the zone of inhibition was distinct. The reactions were as follows: dihydrostreptomycin—inhibition at 100, 10, and 1 μg; polymyxin B—inhibition at 30, 10, and 5 μg; Aureomycin (chlortetracycline)—inhibition at 60, 30, and 10 μg; Chloromycetin (chloramphenicol)—inhibition at 60, 30, and 10 μg; Terramycin (oxytetracycline)—inhibition at 60, 30, and 10 μg; penicillin—inhibition at 10 and 1 μg, no inhibition at 0.5 μg; and bacitracin—no inhibition at 20 and 2 μg.

Gas was evolved when H_2O_2 was added to a loopful of cells placed on the surface of a microscope slide; this indicated the production of catalase.

There was no growth in the presence of 0.001 M KCN when the method described by Edwards and Fife (1) was used.[2]

Taxonomic position of the pathogen.—The walnut canker pathogen is a peritrichic phytopathogenic gram-negative rod and hence falls into the genus *Erwinia.* This genus of phytopathogenic *Enterobacteriaceae* contains 2 main groups, namely, the pectinolytic soft rot species *(E. carotovora)* and members of the group around *E. amylovora.* Because of its inability to degrade pectic substances, its relative "biochemical inertness" in the determinative tests, and its sensitivity to cyanide,[2] the walnut pathogen is closely related to the group around *E. amylovora.*

[2] Sutton, D. D. 1957. Personal communication.

TABLE 1.—*Comparison of the walnut canker pathogen with Erwinia amylovora*

Characteristic	*E. amylovora*[a]	Walnut canker pathogen[a]
Rhamnose fermentation	—	A
Raffinose fermentation	—	A
Cellobiose fermentation	—	A
α-Methylglucoside fermentation	—	A
Glucuronic acid fermentation	—	A
Utilization of citrate	B	—
Voges-Proskauer test	+	— (or very slow +)
Methyl red test	—	+ (reversion to —)
H_2S production	—	+
Virulence to pear	+	—

[a] A = acid formed; B = basic reaction; — = no acid or no reaction; + = positive reaction.

A number of points of difference between the walnut pathogen and authentic *E. amylovora* are summarized in Table 1. As mentioned in a foregoing section, the walnut bacterium does not infect pear, as does *E. amylovora.* The cultural and biochemical differences shown in Table 1 are sufficiently great to justify designation of the walnut pathogen as a new species. We therefore propose the name **Erwinia nigrifluens** sp. nov.

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THE PRODUCTION OF TELIOSPORES OF TILLETIA CARIES IN CULTURE [1]

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SUMMARY

Although *Tilletia caries* (DC.) Tul. is readily cultured in the haploid stage on artificial media, growth in a living host plant has been essential for the production of teliospores. In recent studies with 2 monosporidial isolates from germinating inbred-line spores of race T-16, teliospore production in culture was observed. One of these spore-producing isolates exhibited the normal secondary sporidial growth of haploid lines; the growth covered the face of the agar slant. The masses of teliospores appeared at random on the surface of this growth. In contrast, the only visible growth made by the other isolate was that of the black spore mass. These culture-produced spores germinated like normal teliospores, producing promycelia with whorls of primary sporidia at the tips. Since the dicaryotic stage is required for teliospore production, these teliospore-producing cultures must not have been merely haploid lines. Their behavior suggests that they are either dicaryotic or true diploid lines capable of saprophytic propagation.

Although the smut fungi are readily cultured in the haploid stage on artificial media, growth in a living host plant usually is essential for the production of diploid spores, designated here as teliospores.[3] In some smut fungi, however, teliospore production may occur in culture, this having been reported for species representing 5 genera of the smut fungi, including *Tilletia* (5). In some of the cases reported, the evidence was not conclusive that such culture-produced spores were actually teliospores rather than haploid asexual chlamydospores frequently observed in old cultures of many species of smut fungi.

Sartoris (10) reported spore production by *Ustilago heufleri* Fckl., *U. hordei* (Pers.) Kell. & Sw. and by *U. tritici* (Pers.) Rostr. He germinated these spores and observed that they behaved like those that form on the host plants. Leach and Ryan (9) also showed that the spores produced by *U. striiformis* (West.) Niessl in culture germinated like those produced on the host. If spore germination is a valid criterion for distinguishing between teliospores and chlamydospores, then these reports may be regarded as authentic cases of teliospore production in culture.

In those cases in which spores of *Tilletia caries* (DC.) Tul. (3, 4, 10, 11) and of *T. foetida* (Wallr.) Liro (1) have been reported in culture, the evidence indicates that these were merely haploid chlamydospores, even though they often were morphologically similar to teliospores. Only Sartoris (10) reported on the germination of these culture-produced spores of *T. caries*. Although they had the appearance of teliospores of this species, they did not give rise to the characteristic primary sporidia of germinating teliospores. Instead, they produced mycelium and secondary sporidia only, as would be expected from chlamydospores. Boss (2) observed that spores of *T. caries* produced in artificial culture, even in young stages of development, contained only 1 nucleus, as would be true for the haploid cells that produced chlamydospores. The production of teliospores is dependent on dicaryotic or diploid mycelium. Apparently, therefore, the production of true teliospores in cultures of *Tilletia* spp. has not been recorded heretofore. This paper reports the production in culture of teliospores of *T. caries* from wheat.

MATERIALS AND METHODS.—One pathogenic race of *T. caries* (T-16) was employed in this study. Complete or near complete sets of monosporidial lines for several spores of this race were used. In an attempt to study the inheritance of a sex-associated pigmentation phenomenon (8) occurring in this race, 8 appropriate crosses were made between compatible pigmented and nonpigmented lines. These combinations were inoculated into Red Bobs wheat to obtain spores. Complete sets of primary sporidia were then isolated from germinating spores of this first-generation material. Two of the monosporidial lines obtained from these isolated sporidia produced teliospores in culture.

All monosporidial isolations and inoculations with paired monosporidial lines were made by methods already described (6). Each culture was maintained on potato-sucrose agar and pedigreed by a coded number. Thus, culture 350-1 represents monosporidial line 1 from spore 350; culture 350-2 represents monosporidial line 2 from the same spore, etc. Teliospores resulting from a combination between lines of the same spore were designated as inbred line material (7) and assigned numbers.

RESULTS.—Seven of the 8 compatible combinations that were made produced infection. Teliospores from one of these combinations, (350-2×350-9) designated as inbred line 7, were germinated, and complete sets of primary sporidia were isolated from 24 such spores. The great majority of these isolated sporidia failed to grow in culture; consequently, too few monosporidial lines were obtained for the study of the inheritance

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[3] In this paper, the term teliospore will be used in accordance with Fischer and Holton (5): "Most students of the smut fungi have long perpetuated the use of the term 'chlamydospore' for smut spore. Smut spores are not chlamydospores, but are analogous to teliospores of the rust fungi." The term chlamydospore will be used to refer only to those true asexual spores that do sometimes occur in cultures of smut fungi.

FIG. 1. Teliospore production in a culture of monosporidial line 392-1 of *Tilletia caries*. A) Black teliospore masses appearing at random on the surface of white secondary sporidial growth (×1). B) Close-up of upper 3 teliospore masses seen in A (×8). (Photographs by E. F. Dailey.)

FIG. 2. Teliospore production in a culture of monosporidial line 388-3 of *Tilletia caries*. Note the absence of any secondary sporidial growth on transfer block or adjacent agar surface. Immature spores make up the white area of the smaller teliospore mass located on the transfer block (×10).

of pigmentation. Two of the monosporidial lines obtained from 2 different spores of inbred line 7, however, produced spores in culture. The first monosporidial line (392-1) to give rise to spores in culture was the only one of 25 primary sporidia isolated from spore 392 that grew in culture. The other line (388-3) that produced spores in culture was one of 3 that developed out of 17 primary sporidia isolated from spore 388.

The difference in growth between the 2 spore-producing monosporidial lines was very striking. Line 392-1 (Fig. 1) exhibited a normal type of white secondary sporidial growth covering the face of the agar slant, with the black spore masses appearing at random on the surface of this growth. In contrast, the only visible growth of line 388-3 (Fig. 2) was that of the black spore mass that formed directly on and about the block of agar upon which the primary sporidium had been isolated and transferred to the test tube slant.

To determine whether the spores produced in culture were teliospores, or merely chlamydospores, a portion of the spore mass was removed from the surface of the culture and placed on water agar. These spores germinated like teliospores, producing a promycelium with whorls of primary sporidia at the tip, after only 36 hours at 15°C. Usually the germination of teliospores of *T. caries* requires 4 days or longer, depending on the race.

No experimental evidence is available at this time to explain how these particular monosporidial isolates were able to produce teliospores in culture. Normally, an isolated primary sporidium of *T. caries* gives rise to a haploid monosporidial line. Only in this haploid stage has it heretofore been possible to maintain any *Tilletia* species on artificial media. The dicaryotic stage arises when 2 compatible haploid lines are paired and the sporidia fuse. This constitutes the parasitic stage, which normally grows *in vitro* for a short time only and then dies. Since the dicaryotic stage appears to be a prerequisite for teliospore production, the 2 cultures reported here must not have been merely haploid lines. Their behavior suggests that they were dicaryotic lines capable not only of growing but also of completing the life cycle of the species on a culture medium. It may be significant that only a few of the primary sporidia isolated from spores of this particular parentage were able to grow in culture. Normally a high percentage of isolated primary sporidia of *T. caries* will grow in culture. Thus it seems likely that these teliospore-producing cultures reflect the operation of an unusual genetic pattern for this species.

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A BIOCHEMICAL STUDY OF THE RESISTANCE OF POTATO TUBER TISSUE TO ATTACK BY VARIOUS FUNGI [1]

J. Kuć [2]

SUMMARY

Cephalothecium roseum Cda. and *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr., nonpathogenic to potato tubers, are inhibited by extracts of potato peel. *M. verrucaria*, however, is capable of rotting a peeled tuber and is not inhibited by extracts of incubated tuber tissue, either inoculated or noninoculated. *C. roseum* does not rot a peeled tuber and is inhibited by extracts of inoculated or noninoculated tuber tissue that has been incubated. Two pathogens of potato tubers, *Sclerotium rolfsii* Sacc. and *Fusarium solani* f. *radicicola* (Wr.) Snyd. & Hans. grow well on potato slices and on extracts of potato peel and of incubated tuber tissue, either noninoculated or inoculated. *Aspergillus niger* v. Tiegh., nonpathogenic to potato tubers, also grows well on the various potato extracts; however, spore germination and growth of germ tubes are markedly inhibited on a freshly cut potato slice.

Caffeic and/or chlorogenic acid are present in extracts found to inhibit *C. roseum* and *M. verrucaria*. Both organisms appear more sensitive to inhibition by these acids than do *S. rolfsii*, *F. solani* f. *radicicola*, or *A. niger*. Caffeic and/or chlorogenic acid may be associated with, or responsible in part for, the inhibition of *C. roseum* by extracts of peel and of incubated tuber tissue, either inoculated or noninoculated, and for the inhibition of *M. verrucaria* by peel extracts. The level of chlorogenic and caffeic acid in the various extracts, however, can not account for all the inhibitory activity.

The biochemical factors associated with disease resistance have recently received increased attention. The development of new analytical techniques and recent knowledge of metabolism have provided the impetus for this attention. Though progress has been made in studies on the factors associated with disease resistance in animals, very little is known concerning the nature of disease resistance in plants.

Geissman (1) has reported that cherry leaves infected with rusty mottle virus and peach leaves infected with western X virus contain 2-3 times as much *p*-coumaric acid, esters of *p*-coumaric acid, kampferol, quercitin, caffeic, and chlorogenic acid as do healthy leaves. Kuć et al (3,4) have shown chlorogenic and caffeic acid to be associated with the immunity of white potatoes from attack by Race 1 of *Helminthosporium carbonum* Ullstrup. Potato tuber tissue inoculated with *H. carbonum* produced 2-3 times as much chlorogenic and caffeic acid as did noninoculated control tissue. Peel contained high concentrations of these acids, but here inoculation was unnecessary for their elaboration. Johnson and Schaal (2) and Schaal, Johnson, and Simonds (6) suggested a correlation between the chlorogenic acid content of potato peel and resistance to *Streptomyces scabies* (Thaxt.) Waks. & Henrici. Recently Schaal and Johnson (7) reported autoxidation products of chlorogenic and caffeic acid are more inhibitory to the growth *in vitro* of *S. scabies* than are the parent phenols.

Materials and methods.—*Preparation of tissue extracts.*—For the experiments, Idaho-grown potato tubers (*Solanum tuberosum* L. var. Russet and Netted Gem) free from visible surface injuries were washed with soap and water, surface sterilized by immersion for 2 minutes in aqueous 2.5 per cent sodium hypochlorite, rinsed in sterile water, and then dried. Peel tissue approximately 1 mm thick was removed for extraction, and fresh tuber tissue was obtained

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after removal of the peel. Inoculated tuber tissue was obtained by covering sterile fresh slices, cut approximately 1 cm thick, with a heavy spore and mycelial suspension of the fungus under study. In experiments with *S. rolfsii*, slices were inoculated with hyphal fragments and sclerotia. Slices were incubated for 72 hours in sterilized petri dishes at 72°–76°F in a moist atmosphere. They were then peeled, and a section about 1 mm thick was removed from each inoculated surface for extraction. Autoclaved potato slices were also inoculated, peeled, and prepared for extraction as above; incubated noninoculated tuber tissue was provided by holding sterile potato slices for 72 hours at 72°–76°F in a moist atmosphere, removing the peel, and cutting 1-mm sections from the surface for extraction.

The organisms used were *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr., *Cephalothecium roseum* Cda., *Aspergillus niger* v. Tiegh., *Sclerotium rolfsii* Sacc., and *Fusarium solani* f. *radicicola* (Wr.) Snyd. & Hans. Only the last 2 organisms listed are natural pathogens of white potato tubers.

Extracts were prepared by dropping 40 g of the freshly cut 1-mm tissue sections into 300 ml of boiling 95 per cent ethanol and boiling them for 2–5 minutes. After cooling, the tissue was ground for 5 minutes in a Waring blender, and the homogenate was filtered through Whatman No. 2 filter paper on a Buchner funnel. The filtrate was concentrated to dryness under reduced pressure at 40°C, the residue redissolved in 50 ml of water, and the resulting solution adjusted to pH 5.5. Two g of dehydrated potato-dextrose agar[3] was dissolved in the filtrate, and the solution was autoclaved at 15 lb. pressure for 10 minutes. Petri dishes containing 16 ml of the sterile nutrient medium were seeded centrally with a drop of a dilute spore and mycelial suspension of the fungus under study. Hyphal fragments and sclerotia were used to seed petri dishes in experiments with *S. rolfsii*. Fungi were incubated at 72°–76°F except that *S. rolfsii* was incubated at 82°F.

Spectral absorption and chromatographic analysis of crude potato extracts.—Tissue extracts were prepared as described and concentrated to 100 ml at 40°C under reduced pressure. Aliquots of the extracts were diluted with an appropriate volume of 95 per cent ethanol and examined spectrophotometrically (Beckman DU) in the range of 250–350 mμ.

The tissue extracts used for spectral analysis were employed for chromatographic separation on paper. Approximately 50 λ of each of the extracts was applied with a capillary pipette in spots 2 cm apart on 18¼ × 22½-in. sheets of Whatman No. 1 filter paper. Chromatograms were developed at 25°C for 16–18 hours, with 1-butanol, acetic acid, and water (4:1:1) used as descending developing solvent. The papers were dried for 6–8 hours, observed under ultraviolet radiation, and then sprayed with either Roberts and Wood reagent (5), diazotized sulfanilic acid,[4] ammoniacal silver nitrate,[5] or 1 per cent ferric chloride to determine the location of phenolic compounds.

Preparation of media containing chlorogenic or caffeic acid.—To a medium containing 4 g of dehydrated potato-dextrose agar dissolved in 100 ml of an extract of fresh tuber tissue, prepared as described earlier, was added chlorogenic or caffeic acid. The medium was buffered at pH 5.5 by the pulp extract and the potato-dextrose agar. Petri dishes containing 16 ml of the sterile medium were seeded with the fungus under study. Hyphal fragments and sclerotia were used to seed dishes with *S. rolfsii*, and a spore and mycelial suspension was used for the other fungi. Control medium containing potato-dextrose agar dissolved in tuber extract was similarly seeded. Fungi were incubated at 78°–82°F, and the colony diameters were measured at 24-hour intervals.

RESULTS.—*Growth of fungi on potato tuber tissue.*—All fungi made good growth on autoclaved potato slices. *A. niger* and *C. roseum* made little or no growth on living potato slices; *F. solani* f. *radicicola* made fair growth, and *M. verrucaria* and *S. rolfsii* completely rotted the slice surface. It is interesting to note that *M. verrucaria*, normally not a pathogen of potatoes, was capable of rotting the peeled tuber. Though *C. roseum* did not grow on potato slices, spore germination was normal on the slice surface; however, spore germination of *A. niger* was markedly retarded and germ tubes were stunted.

Growth of fungi on extracts of potato tissues.—The fungi made good growth in 5 days on both the dehydrated potato-dextrose agar medium and on the agar medium to which the extract of fresh potato tuber tissue or the extract of autoclaved inoculated tuber tissue had been added (Table 1). *M. verrucaria*, *A. niger*, *S. rolfsii*, and *F. solani* f. *radicicola* made good growth on the medium to which an extract of incubated noninoculated tuber tissue had been added, whereas *C. roseum* was inhibited on this medium. *M. verrucaria* and *C. roseum* were inhibited on the medium containing the extract of peel, but *A. niger*, *S. rolfsii*, and *F. solani* f. *radicicola* made good growth on this medium. An extract of tuber tissue inoculated with *C. roseum* was inhibitory to *C. roseum*, but extracts of tissue inoculated with the other fungi were not inhibitory to the specific fungus used to inoculate the slice surface.

[3] Difco, Difco Laboratories, Inc., Detroit, Michigan.

[4] Fifty g of sulfanilic acid is dissolved in 250 ml 10 per cent KOH and the solution is cooled. To this is added 200 ml 10 per cent $NaNO_2$. The resulting solution is added slowly to an ice-cold HCl solution (80 ml concentrated HCl plus 40 ml water), and the precipitated diazonium salt is filtered off with ice water, washed with absolute ethanol and ether, and dried in air. Immediately before spraying, 0.1 g of the diazonium salt is dissolved in 20 ml of 10 per cent aqueous Na_2CO_3. Phenolic compounds appear as yellow, red, or brown spots.

[5] Eight ml of concentrated NH_4OH and 17 ml of water are added to 25 ml of 0.1 N $AgNO_3$ solution. Phenolic compounds appear immediately as reddish-brown or gray spots.

TABLE 1.—*Growth of Cephalothecium roseum, Myrotheciu n verrucaria, Aspergillus niger, Sclerotium rolfsii and Fusarium solani f. radicicola on potato-dextrose agar containing extracts of various potato tissues*

Extract added to potato-dextrose agar	Colony diameter (mm) after 120 hours[a]				
	C. roseum	*M. verrucaria*[b]	*A. niger*	*S. rolfsii*	*F. solani* f. *radicicola*[c]
None	65	30	79	90	20
Fresh tuber tissue	70	34	82	90	19
Peel	17(R)	22(R)	85	90	17
Autoclaved inoculated tuber tissue	66	31	72	90	18
Incubated noninoculated tuber tissue	22(R)	31	80	90	18
Inoculated tuber tissue[d]	20(R)	31	79	90[e]	18

[a] (R) indicates red pigmentation.
[b] After 240 hours, colony diameter was 80 mm on all media except those containing extracts of incubated noninoculated tuber tissue or of peel on which it was 65 mm and 30 mm, respectively.
[c] After 240 hours, colony diameter of *F. solani* f. *radicicola* was 45 mm on all media except potato-dextrose agar, on which it was 50 mm.
[d] In each case the fungus used to inoculate the tuber slice was the same as that for which growth data are given.
[e] Reaction of this was pH 3. *S. rolfsii* grows at this pH and at pH 5.5.

A red halo developed around colonies of *C. roseum* growing on media containing an extract of peel, of incubated noninoculated tuber tissue, or of inoculated tuber tissue. A similar halo developed around colonies of *M. verrucaria* growing on an extract of peel. The halo appeared 48 hours after inoculation; after its formation, little or no further growth occurred.

Spectral absorption and chromatographic analysis of crude potato extracts.—Reagent grade chlorogenic acid, reagent grade caffeic acid, extracts of potato peel, extracts of incubated noninoculated tuber tissue, and extracts of tissue inoculated with *A. niger*, *C. roseum*, *S. rolfsii*, or *F. solani* f. *radicicola* showed substantial absorption in the range of 280–330 mμ (Figures 1, 2). The extracts of peel and of tuber tissue inoculated with *F. solani* f. *radicicola* exhibited a higher absorbance in this range than did the other extracts. With extracts of fresh tuber tissue, of tissue inoculated with *M. verrucaria*, and of autoclaved tissue inoculated with either *C. roseum*, *M. verrucaria*, *S. rolfsii*, or *F. solani* f. *radicicola*, the absorption maximum was at 270 mμ and absorption at 280–330 mμ was slight. The absorption spectrum of the extract of autoclaved tissue inoculated with *A. niger* was similar to, but at a higher level than, that of extracts of autoclaved tissue inoculated with the other fungi. None of the extracts of inoculated autoclaved tissue inhibited growth of the fungus used for inoculation.

Chromatographic separation of the extracts of peel and tuber tissue inoculated with *F. solani* f. *radicicola* showed the presence of 2 strongly fluorescent compounds, with R_f values of 0.45 (Compound 1) and 0.72 (Compound 2). Extracts of incubated noninoculated tuber tissue and of tissue inoculated with *C. roseum*, *A. niger*, or *S. rolfsii* appeared to contain Compound 1 but little or no Compound 2.

Compounds 1 and 2, previously found in extracts of potato peel, have been identified as chlorogenic and caffeic acid, respectively (4). The fluorescent material present in extracts of incubated noninoculated tuber tissue and of tissue inoculated with *C. roseum*, *A. niger*, *S. rolfsii*, or *F. solani* f. *radicicola* has an R_f value the same as that of chlorogenic acid[6] or of the chlorogenic acid separated from potato peel extracts. The materials also gave identical color reactions. Compound 2 present in an extract of tuber tissue inoculated with *F. solani* f. *radicicola* has an R_f value identical with that of caffeic acid[6] or of caffeic acid separated from potato peel. The color reactions of the materials also are identical. The concentration of Compound 1 appeared highest in extracts of peel and in slices inoculated with *F. solani* f. *radicicola* and lowest in extracts of incubated noninoculated slices. Two to 3 faintly fluorescent compounds, R_f 0.75–0.90, were observed on chromatograms of extracts of peel and of tuber tissue inoculated with *A. niger*, *S. rolfsii*, *F. solani* f. *radicicola*, or *C. roseum*. These compounds gave a red color when sprayed with diazotized sulfanilic acid but did not reduce ammoniacal silver nitrate.

Extracts of fresh tuber tissue, of autoclaved tissue inoculated with any of the fungi studied, and of tissue inoculated with *M. verrucaria* contained none of these compounds in appreciable amounts if at all.

Growth of fungi on media containing either chlorogenic or caffeic acid.—*S. rolfsii*, *F. solani* f. *radicicola*, and *A. niger* grew well on the media containing 5×10^{-3} M or 1×10^{-2} M chlorogenic acid or 5×10^{-3} M caffeic acid (Table 2). Growth was normal, except that it was inhibited in the medium containing 1×10^{-2} M caffeic acid. The growth of *C. roseum* or of *M. verrucaria* was inhibited on media containing 10^{-2} M chlorogenic acid or 5×10^{-3} M caffeic acid and almost completely stopped on media containing 10^{-2} M caffeic acid. Red pigmentation was evident around colonies of *M. verrucaria* growing on media containing either chlorogenic or caffeic acid. The lack of red pigmentation around colonies of *C. roseum* suggests that other factors, in addition to the presence of chlorogenic acid or of caffeic acid, are associated with

[6] Reagent grade, Mann Research Laboratories, Inc., New York 8, N. Y.

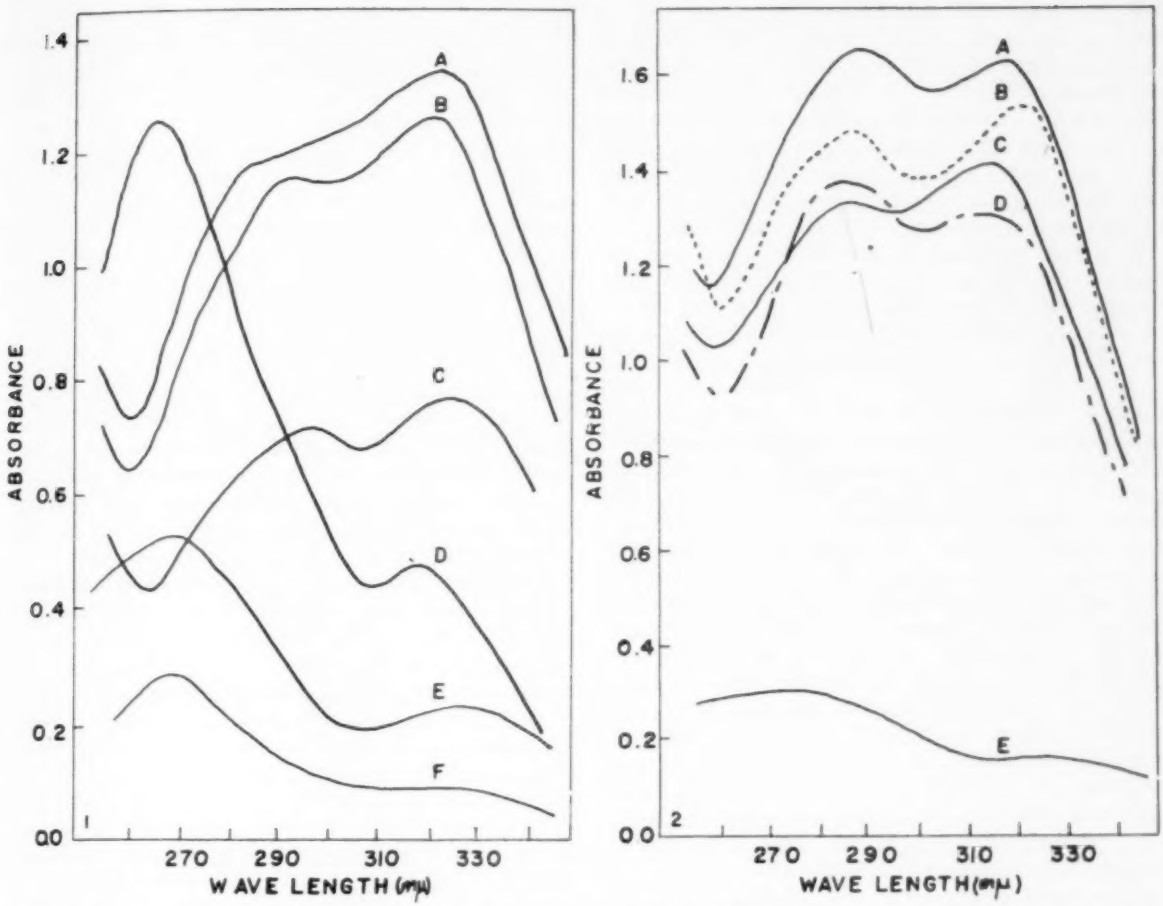

FIG. 1–2. FIG. 1. Spectral absorption curves of chlorogenic acid, of caffeic acid, and of different tuber extracts. Except as noted, 2-ml aliquots of extracts corresponding to 0.8 g of potato tissue were diluted with 18 ml of 95 per cent ethanol. A) Chlorogenic acid (or of caffeic acid). B) Peel. One-ml aliquot, corresponding to 0.4 g of potato tissue, diluted with 18 ml 95 per cent ethanol. C) Noninoculated tuber tissue held for 72 hours. D) Tuber tissue autoclaved then inoculated with *Aspergillus niger*. E) Tuber tissue autoclaved then inoculated with either *Cephalothecium roseum*, *Myrothecium verrucaria*, *Sclerotium rolfsii*, or *Fusarium solani* f. *radicicola*. F) Fresh tuber tissue. —FIG. 2. A) Tuber tissue inoculated with *A. niger* and incubated for 72 hours at 22° C. B) Tissue inoculated with *S. rolfsii* and incubated. C) Tissue inoculated with *F. so'ani* f. *radicicola* and incubated. One-ml aliquot, corresponding to 0.4 g of potato tissue, diluted with 18 ml of 95 per cent ethanol. D) Tissue inoculated with *C. roseum* and incubated. E) Tissue inoculated with *M. verrucaria* and incubated.

the production of red pigmentation by this fungus.

DISCUSSION.—The mechanism responsible for the resistance or susceptibility of a plant tissue to a specific fungus is exceedingly complex and appears dependent on the fungus used for inoculation. It has been reported (3) that *H. carbonum*, *Ceratostomella ulmi*

TABLE 2.—*Growth of fungi on potato-dextrose agar containing various concentrations of chlorogenic acid and of caffeic acid*

Fungus	Colony diameter on media containing indicated acid*				
	None	Chlorogenic acid		Caffeic acid	
		5×10^{-3} M	10^{-2} M	5×10^{-3} M	10^{-2} M
Cephalothecium roseum	55	48	45	29	0
Myrothecium verrucaria	30	28(R)	24(R)	23(R)	(T)
Aspergillus niger	90	90	90	90	74
Sclerotium rolfsii	90	90	90	90	60
Fusarium solani f. *radicicola*	25	24	25	25	10

* All media contain 4 g dehydrated potato-dextrose agar dissolved in 100 ml of an extract of fresh tuber tissue. The media were buffered by the ingredients at pH 5.5. (R) Indicates red pigmentation, (T) indicates trace of growth.

Buis. and *Fusarium oxysporium* f. *lycopersici* (Sacc.) Snyd. & Hans. are strongly inhibited by extracts of potato peel and by extracts of tuber tissue previously inoculated with any of the 3 fungi but only slightly inhibited by extracts of incubated noninoculated tuber tissue. These organisms are not known to incite disease in potato tubers. Data in this paper suggest that *M. verrucaria*, *C. roseum* and *A. niger* are nonpathogenic to potato tubers for different reasons.

C. roseum resembles *H. carbonum* in that both are inhibited by extracts of peel and of inoculated tuber tissue; however, they differ in that *C. roseum* also is inhibited by an extract of incubated noninoculated tissue. *M. verrucaria* either does not incite production of an inhibitory substance after inoculation or detoxifies it if produced. The fungus rots a cut slice and is not inhibited by an extract of inoculated tuber tissue. The fungus is, however, inhibited by an extract of peel. It appears that the only defense a potato tuber possesses against *M. verrucaria* is passive—the presence of an inhibitor in the peel, the first barrier to infection. *A. niger* grows well on extracts of potato peel and on incubated tuber tissue, either inoculated or noninoculated. Spore germination and growth of germ tubes, however, are markedly inhibited on the surface of a freshly cut potato slice. The growth of the 2 potato pathogens *S. rolfsii* and *F. solani* f. *radicicola* appears to be unaffected by any defense mechanism of the tuber. They grow well on extracts of peel and of incubated tuber tissue, either inoculated or noninoculated, and on the tuber itself.

Chlorogenic and caffeic acid have been reported present (4) in extracts of potato peel of incubated noninoculated tuber tissue and of tissue inoculated with *H. carbonum* and to be associated with the inhibitory activity of these extracts toward *H. carbonum*. Spectral analyses and paper chromatography suggest the presence of chlorogenic acid, and often caffeic acid, in extracts inhibitory to *C. roseum* and *M. verrucaria* and their absence or presence in low concentration in noninhibitory extracts. Chlorogenic and caffeic acid may be associated with or responsible in part for the inhibitory activity of extracts of peel, of inoculated tuber tissue, and of incubated noninoculated tissue toward *C. roseum* and of peel extracts toward *M. verrucaria*. The level of chlorogenic and caffeic acid in various extracts, however, can not account for all the observed inhibitory activity.

The ability to produce chlorogenic and/or caffeic acid, therefore, does not solely determine the resistance of potato tubers to disease. Both acids are produced by tuber tissue inoculated with *F. solani* f. *radicicola* and chlorogenic acid by tissue inoculated with *S. rolfsii*, yet these fungi parasitize potato tubers. Studies with reagent grade chlorogenic and caffeic acids indicate that the 2 potato pathogens appear to tolerate a higher concentration of the acids than do *C. roseum* and *M. verrucaria*. Spore germination and growth of *A. niger* appear inhibited on freshly cut potato slices before there has been an accumulation of chlorogenic and caffeic acid, and this organism, like the 2 potato pathogens, is not sensitive to a high concentration of the acids.

The production of a red halo around colonies of *C. roseum*, *M. verrucaria*, and *H. carbonum* growing on potato extracts high in chlorogenic and caffeic acid suggests oxidation of the phenolic compounds by an extracellular oxidase. Whether oxidation products or their parent phenols are active inhibitors is still uncertain, and the significance of the red pigment formation is being investigated.

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THE GRASSHOPPER AS A VECTOR OF TOBACCO RINGSPOT VIRUS IN SOYBEAN[1]

John M. Dunleavy[2]

SUMMARY

Tobacco ringspot virus was transmitted from tobacco to soybean and from soybean to soybean by *Melanoplus differentialis* (Thos.) and by a mixture of *M. mexicanus* (Sauss.) and *M. femur-rubrum* (De G.). The insects were starved for 4 hours and then allowed to feed approximately 1 minute on diseased plants before they were transferred to healthy plants. The length of time such grasshoppers fed on healthy soybean plants had a marked effect on percentage of plants infected. The greatest percentage of infected plants was obtained when insects were allowed to take only 1 bite of leaf tissue. No transmission of the virus was obtained when insects were allowed to feed longer than 0.5 minute. Because of the feeding habits of grasshoppers and the manipulation of the insects on soybean plants necessary to obtain only 1.2 per cent infection, they are not considered an important vector of tobacco ringspot virus.

Bud blight disease of soybean (*Glycine max* (L.) Merr.) is caused by tobacco ringspot virus (1) and is among the most destructive of the viruses infecting soybean. In young plants, the virus may kill the upper portion of the main stem, including the flower buds. If older plants are infected, only small, poorly developed pods are formed, many of which may fall to the ground. The virus occurs in soybean largely in the midwestern United States and in Ontario, Canada. Between 1943 and 1947, losses were reported ranging from 25 to 100 per cent in the Midwest (7). From 1949 to 1954, the disease was reported to occur sporadically in trace amounts with only a very few notable exceptions. Records of disease surveys in Iowa from 1953 to 1956 show that bud blight generally was found only in trace amounts but that the number of fields in which the disease was reported increased during this period (4, 5). Hildebrand and Koch (6) reported no significant difference in the incidence of bud blight when seed from virus-infected plants was compared with seed from healthy plants; however, Desjardins et al (3) have reported seed transmission of tobacco ringspot virus in the Lincoln variety of soybean grown in the greenhouse.

Although seed transmission may explain the source of inoculum for current-season spread of the virus, it does not explain why the disease has not been observed in the field prior to the first week in July (1, 6). Allington (1), Chamberlain and Koehler (2), Johnson et al (7), and the author have observed that the disease usually appears at the edges of fields and advances inward as the season progresses. Such observations suggest the presence of an insect vector. Since the grasshopper has been shown by Walters (8) to transmit tobacco ringspot virus from tobacco (*Nicotiana tabacum* L.) to tobacco, it was decided to determine if the virus could be transmitted from tobacco to soybean and from soybean to soybean by this vector.

MATERIALS AND METHODS.—All work was conducted in the greenhouse. The 3 species of grasshopper used were *Melanoplus differentialis* (Thos.), the differential grasshopper; *M. mexicanus* (Sauss.), the migratory grasshopper; and *M. femur-rubrum* (De G.), the red-legged grasshopper. Insects were collected in the fall and maintained in cages until sufficient egg pods were obtained. Egg pods were stored in moist sand at 5°C and incubated at room temperature as needed. All insects used in this study were obtained from eggs and were fed soybeans and corn.

Insects were starved for 4 hours prior to use in transmission experiments. After insects had fed on diseased plants for approximately 1 minute, they were placed on healthy plants; care was taken that only the insect made contact with the plant. Similar care was used in removing insects from plants after they had fed.

Soybean plants 30–40 days old were used throughout the study. In all cases of grasshopper transmission of the virus to soybean, the presence of tobacco ringspot virus in the test plants was confirmed by mechanical transmission to cowpea (*Vigna sinensis* (L.) Endl.), petunia (*Petunia hybrida* Hort.), and tobacco.

EXPERIMENTAL RESULTS.—Budblight of soybean was observed most frequently during the years 1953–56 in southern Iowa, where heavy infestations of *M. differentialis* occurred. The disease was observed less frequently in central and northern Iowa, where a mixture of *M. mexicanus* and *M. femur-rubrum* constituted the predominant grasshopper population. Initial transmission trials, in which *M. differentialis* or a mixture of *M. mexicanus* and *M. femur-rubrum* were allowed feeding periods of 5 minutes, were unsuccessful. The insects would eat large portions of the leaflets if allowed to feed more than 1 or 2 minutes. An insect would usually begin feeding at the margin of a leaflet and eat along the edge in a forward direction for a few millimeters then return to the original starting point and eat the leaf tissue previously contacted by the mouth parts. This procedure was usually repeated several times before the grasshopper would move to a new location on a leaflet. These observa-

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[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and Iowa Agricultural Experiment Station.

tions suggested that grasshopper transmission of the virus might be obtained if the insects were allowed to feed for only a short time on healthy plants to prevent them from eating previously inoculated leaf tissue.

M. differentialis was used in another experiment to determine the effect on virus transmission of length of active feeding time on healthy soybean plants after the grasshoppers had previously fed on virus-infected soybean plants. Grasshoppers were fed on healthy plants for periods ranging from 0.5 to 3 minutes. In addition, some grasshoppers were allowed to take only 1 bite of leaf tissue before being removed. The virus was not transmitted when the insects were allowed to feed for periods longer than 0.5 minute (Table 1). When fed for 0.5 minute, 0.7 per cent of the grasshoppers transmitted the virus. When the insects were allowed to take only 1 bite of leaf tissue, however, 2.0 per cent of them transmitted the virus. Results of the test indicate that transmission probably results from mechanical transfer of sap from infected to healthy plants on grasshopper mouth parts. It seems probable that much of this sap would be wiped off the mouth parts, or be otherwise depleted, during the first few bites of leaf tissue that the insect takes from a healthy plant. Because of the feeding habits of grasshoppers, leaf tissue that might have been inoculated during the first few bites is subsequently eaten by them. In all remaining transmission trials, grasshoppers were allowed to take only 1 bite from healthy plants.

Attempts to transmit tobacco ringspot virus from tobacco to soybean with *M. differentialis* and with a mixture of *M. mexicanus* and *M. femur-rubrum* were successful (Table 1). *M. differentialis* transmitted the virus in 2.3 per cent of the cases, whereas the mixture transmitted the virus in 1.2 per cent of the cases.

TABLE 1.—*Percentage of soybean test plants infected after being fed upon for various periods of time by grasshoppers that previously had been starved for 4 hours and then fed for about 1 minute on soybean or tobacco plants infected with tobacco ringspot virus*

Grasshopper species	Source plant	Active feeding time on test plants	No. of trials	Percentage of soybean plants infected
Melanoplus differentialis	Soybean	3 Minutes	150	0
		2 Minutes	150	0
		1 Minute	150	0
		0.5 Minute	150	0.7
		1 Bite	150	2.0
M. differentialis	Tobacco	1 Bite	310	2.3
M. mexicanus plus *M. femur-rubrum*	Tobacco	1 Bite	245	1.2
M. differentialis	Soybean	1 Bite	250	1.2
M. mexicanus plus *M. femur-rubrum*	Soybean	1 Bite	250	0.4

The virus was successfully transmitted from soybean to soybean in 1.2 per cent of the cases when *M. differentialis* was used and in 0.4 per cent of the cases when the mixture of *M. mexicanus* and *M. femur-rubrum* was used.

In the transmission trials from tobacco to soybean and from soybean to soybean, *M. differentialis* was the more efficient vector, transmitting the virus with about twice the frequency of the mixed population. In this instance, vector efficiency could be related to insect size. *M. differentialis* is almost twice as large as either *M. mexicanus* or *M. femur-rubrum* and could carry more infective sap on its mouth parts than could either of the smaller grasshoppers.

Grasshopper populations in Iowa soybean fields may reach 50,000 per acre in years when infestations are severe. In other years, populations are much smaller. The insects tend to feed several minutes on a plant unless disturbed and may feed on 1 plant entirely. After feeding, they usually move to other plants to rest. The extent to which grasshoppers transmit tobacco ringspot virus under field conditions would depend upon insect population, the number of infected plants present, and how frequently insects are disturbed during feeding. If grasshopper population and the number of virus infected plants are high, and the insects are frequently disturbed during feeding, they could be important in spread of the virus causing bud blight; however, such a situation would be extremely rare. In recent years, bud blight has very rarely been reported on more than 1 per cent of the plants in a given field; in 4 years of observing the disease in Iowa, the author has never observed more than 0.01 per cent infection in July. When the feeding habits of grasshoppers and the manipulations necessary to obtain a very small percentage of transmissions of the virus are considered, it does not appear that the grasshopper is an important vector in the transmission of tobacco ringspot virus from infected to healthy soybean plants.

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PROPERTIES OF CERTAIN MECHANICALLY TRANSMITTED VIRUSES OF PRUNUS[1]

Robert W. Fulton

SUMMARY

In sodium phosphate buffer extracts of infected cucumber cotyledons or squash leaves, Prunus viruses A, E, and G were most infective at pH 8.0 whereas virus B was most infective at pH 8.7. The infectivity of all 4 viruses in 0.1 M buffer was much less than that in 0.01 or 0.03 M buffer. Thermal inactivation in 10 minutes occurred at 56°C with viruses E and G, at 54° with virus A, and at 44° with virus B, all of which were tested in 1:25 dilutions in buffer. In similar extracts held at 24°, virus A remained infective for 6 hours, virus G for 9 hours, and viruses B and E for 15 hours. Leaves rinsed with water after inoculation developed only 10 to 30 per cent as many lesions as did leaves blotted dry without rinsing. In undiluted sap, all 4 viruses lost most of their infectivity in less than 2 minutes. In dilute (1:80) extracts, viruses A, E, and G lost more than half the original infectivity within 1 hour at 24°. Virus B was more stable than the others, 1:80 extracts retaining nearly half their infectivity for 3 hours. Extracts containing viruses A, E, and G lost more than half their infectivity in 3 hours at 0°, whereas virus B extracts lost no infectivity. Cysteine hydrochloride or other reducing agents did not prolong infectivity of any of the 4 viruses. Viruses B and E in extracts containing 0.001 M NaCN lost infectivity less rapidly than in buffer alone. The infectivity of all 4 viruses was markedly decreased by 0.01 M $CaCl_2$ or by other salts containing divalent metallic ions. Virus B was more infectious in 0.03 M sodium oxalate than in phosphate buffer, but oxalate inhibited the infectivities of viruses A, E, and G. In buffer extracts containing 0.01 M cysteine hydrochloride and either 0.001 M NaCN or 0.01 M sodium oxalate, virus B lost little or no infectivity in 3 hours. These combinations were completely ineffective in stabilizing viruses A, E, and G. On the basis of property differences it is concluded that virus B is different from A, E, or G. Virus E may be distinct from A or G, whereas these 2 are very similar in their properties.

Four viruses, A, B, E, and G, which were transmitted to cucumber (*Cucumis sativus* L.) from naturally infected sour cherry (*Prunus cerasus* L.), have been differentiated on the basis of herbaceous host reactions (3). Differences in host ranges have provided a convenient method of separating these viruses. Local-lesion hosts suitable for quantitative assay also have been found and were used in the present work on properties of the viruses.

The first objective in these property studies was to determine whether viruses A, B, E, and G could be distinguished on the basis of property differences *in vitro*. Since virus strains may vary in host range, it was thought that differences or similarities in properties might provide additional evidence for or against differentiation of the 4 viruses. Another objective was to devise methods for obtaining maximum infection with the viruses. Most attempts to transmit viruses mechanically to *Prunus* spp. have been unsuccessful, possibly in part because of the use of inoculum of low infectivity. Since preliminary trials (4) showed that viruses A, B, E, and G were markedly unstable in extracts, part of the work reported here represents attempts to increase stability and infectivity. It seemed likely that if the viruses were to be identified as the causal agents of specific stone fruit diseases, mechanical transmission to the original Prunus hosts might succeed if more highly infective inoculum were used than had been available previously.

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MATERIALS AND METHODS.—In the determination of the effect of various treatments on relative infectivity, assays of viruses A, E, and G were made on primary leaves of *Momordica balsamina* L. In some trials with virus E, guar (*Cyamopsis tetragonoloba* (L.) Taub.) was used as an assay host, but it was unsatisfactory under long-day conditions. *Sesbania exaltata* (Raf.) Cory was used as an assay host for virus B. Lesions induced on these hosts have been illustrated previously (3).

Infectious extracts of viruses A, E, and G were prepared by grinding tissue from inoculated cucumber cotyledons (*Cucumis sativus* L. var. Chicago Pickling) in buffer or other diluents. As has been pointed out (3), the amount of active virus in these cotyledons rose to a high level within 4 or 5 days after inoculation. This level was maintained for only a few days, then dropped rapidly. Nine to 12 days after inoculation the infectivity of cotyledon extracts was low. Extracts from systemically infected cucumber leaves were less infectious than extracts from locally infected cotyledons.

Seedling cucumbers to be used for inoculum were inoculated with extracts of cotyledons that had been infected for 5 to 7 days and were showing pronounced yellowing. Because heavily inoculated cotyledons tended to become necrotic during short days, all plants to be used for inoculum were placed about 1 ft. below a bank of three 40-watt fluorescent lights operated to give a 20-hour day and were shielded from direct sunlight. In spite of this treatment, variations in inoculum at different times of the year apparently were not eliminated. Lower dilutions were required to

induce a given number of lesions in the spring and fall than during the winter. This might also have been due to changes in the susceptibility of assay plants. The failure to increase or decrease the virus dilution in response to these changes has resulted in variations in total lesions produced in different replications of the same experiment at different times. The relative effects of different treatments within a trial appeared to be the same for large as for small numbers of total lesions.

Inoculum of virus B was prepared from systemically infected leaves of squash (*Cucurbita moschata* Duchesne var. Butternut). After invasion by virus B, squash leaves turned a nearly uniform bright yellow and provided uniformly infectious inoculum for several weeks.

In preliminary trials it was found that the 4 viruses in undiluted sap lost most of their infectivity within 2 minutes. Inoculum was prepared, therefore, by thoroughly grinding tissue directly in the diluent. Disks of tissue were punched from infected cotyledons or leaves with a 4-mm cork borer. One hundred disks of fresh cotyledon tissue, taken midway between the center and margin, weighed about 0.4 g. In the computation of dilutions, 10 disks were considered the equivalent of 0.04 ml of sap, thus 10 disks ground in 1.0 ml gave approximately a 1:25 dilution. The thinner squash leaf disks weighed about 0.2 g per 100 disks. Dilutions required to give significant numbers of lesions varied from about 1:100 in winter to 1:25 in late spring.

Most trials involving viruses A, E, and G were designed so that 4 treatments were assigned to opposite halves of the 2 primary leaves of *M. balsamina*, with 3 plants used for each trial. In most trials with virus B, 4 treatments were paired in all possible combinations on the opposite cotyledons of 12 plants of *S. exaltata*.

Inoculations were made by wiping Carborundum-dusted leaves with a small cheesecloth pad saturated with inoculum of viruses A, E, or G. The small cotyledons of *S. exaltata* were more conveniently inoculated with virus B by means of a ground-glass spatula. All plants were grown in a greenhouse maintained at about 24°.

RESULTS.—*The effect of pH and buffer concentration on infectivity.*—In the comparison of the effect of pH on infectivity, tissue disks were ground in 0.03 M sodium phosphate buffer at pH 6.0, 7.0, 8.0, 8.7, and (with virus B) 9.4. Inoculations were made immediately after the tissue was ground. The results (Table 1) showed that pH 8.0 was optimum for infection by viruses A and G. Virus E was equally infectious at pH 7.0 and pH 8.0. Virus B, however, was most infectious at pH 8.7. All subsequent trials were made at pH 8.0 with viruses A, E, and G and at pH 8.7 with virus B, except where otherwise noted.

Use of 0.03 M buffers resulted in the greatest infectivity, except with virus B, which was slightly more infectious at a buffer concentration of 0.01 M than at any other (Table 1). A buffer concentration of 0.03 M was used in subsequent trials with all viruses. In 0.1 M buffer, the infectivity of all viruses was greatly reduced. A similar effect has been reported by Lindner et al (5), who noted that their stone fruit virus in cucumber extract was denatured by high salt concentrations.

TABLE 1.—*Infectivity of 4 Prunus viruses when infected cucurbit tissue was ground in buffers at various pH values and concentrations and used immediately for inoculation of local-lesion hosts*

Buffer		No. of lesions with the following viruses[a]			
Molarity	pH	A	B	E	G
0.03	6.0	20	36	109	121
	7.0	35	46	183	147
	8.0	52	58	181	170
	8.7	26	92	102	120
	9.4		16		
0	8.0[b]	19	91	170	3
0.01	8.0[b]	16	104	343	15
0.03	8.0[b]	53	89	356	105
0.1	8.0[b]	3	15	45	2

[a] Values are averages of total lesions on 3 half-leaves in each of 3 trials.

[b] pH 8 buffer was used for all except virus B, for which the buffer was at pH 8.7.

Resistance to heat and to aging in vitro.—Thermal inactivation trials were run at the optimum pH with tissue ground in sufficient 0.03 M phosphate buffer to give a dilution of 1:25. Since it was known that the viruses lost infectivity rapidly, each sample was ground separately just before it was heated for 10 minutes; it was used for inoculation immediately after being cooled. In these trials, infectivity was determined by inoculation to cucumber plants. Temperatures inactivating viruses A, E, and G, based on 3 or 4 replications, were essentially the same, virus A being inactivated at 54° but not at 52°, whereas viruses E and G were inactivated at 56°. A markedly lower inactivation temperature of 44° was found for virus B.

Aging tests were run at 24° with 1:25 extracts in 0.03 M buffer at pH 8.0 or 8.7, and infectivity was tested by inoculation of cucumber plants. In 3 trials with each virus, consistent differences were found. Virus A was completely inactive in 9 hours but not in 6, whereas virus G was inactive after 12 hours but not after 9 hours. Viruses B and E survived 15 hours but not 18.

In these trials, the pH and buffer concentration were optimum for infectivity but may not have been optimum for heat tolerance or longevity. Also, it is likely that dilution might have affected the observed end points. The primary objective, however, was to compare the viruses, and on the basis of these tests, the viruses showed differences in stability. Boyle et al (1) have reported a thermal inactivation of 52° and a resistance to aging of less than 12 hours for a cherry virus in cucumber. The present results seem to be in close agreement with their determinations, which were

made on undiluted cucumber sap. Figures slightly higher than these were reported by Weintraub and Willison (8), who used 1:10 dilutions of cucumber extracts of several stone fruit viruses.

Effect of postinoculation treatment of leaves on lesion numbers.—It was shown by Yarwood (9) and confirmed by several other investigators that leaves rinsed with water after inoculation may develop markedly fewer primary lesions than do comparable nonrinsed leaves. This was found to be the case with the 4 viruses used in this investigation. Leaves or cotyledons rinsed with water immediately after inoculation and kept wet for 15 or 20 minutes developed only 10–30 per cent as many lesions as did leaves blotted dry immediately after inoculation. Rinsing was less detrimental than this if leaves were blotted dry immediately, but even with this treatment numbers of lesions were only 50–85 per cent as great as on the nonrinsed control. Rinsing with 0.03 M phosphate buffer at pH 8.0 or 8.7, although less detrimental than rinsing with water, resulted in lower lesion counts than did leaving the plants nonrinsed. All inoculations, therefore, were made without rinsing. Excess liquid could usually be drawn from the surface of the leaf with the cloth used for inoculation, or with the spatula, so that the surface of the leaf dried very soon after inoculation. This procedure was used routinely since it was faster than blotting, and each half-leaf in an assay series dried rapidly and uniformly.

Factors affecting aging in vitro.—Preliminary trials showed that inoculum prepared from undiluted cucumber sap had very low infectivity. Attempts were made to determine if this was due to a detrimental effect of the cucumber sap on the assay hosts or to a rapid inactivation of virus. In trials with each of the 4 viruses, uniform batches of tissue were ground and buffer then added to each after 10, 30, or 90 seconds (final dilution–1:80). The infectivities of these preparations were compared with the infectivity of an extract made by grinding tissue directly in buffer. The results (Table 2) showed a very rapid inactivation of virus in undiluted sap.

The rate of loss of infectivity of all viruses in undiluted sap occurred so rapidly that differences among them may not have been detected. Quantitative comparisons of rate of infectivity losses at 24°C were made, therefore, on 1:80 dilutions at hourly intervals over a 3-hour period. More than half the infectivity of viruses A, E, and G was lost within the first hour after preparation of the extracts (Table 3); most of the original infectivity was lost at the end of 3 hours. Virus B lost infectivity less rapidly than did the others. At the end of 2 hours, approximately one-half the original infectivity remained; after 3 hours extracts containing virus B were much more infective than those containing the other viruses. Since virus B was tested at pH 8.7, whereas viruses A, E, and G were tested at pH 8.0, it seemed possible that the difference might have been due to pH; however, other trials with virus B showed that infectivity was lost more rapidly at pH 7.0 and 8.0 than at pH 8.7.

Virus stability at 0°C was determined by preparing extracts in mortars precooled and packed in snow or ice during the 3-hour period of the tests. Only virus B did not lose infectivity at 0° (Table 3). Surprisingly, viruses A, E, and G lost 60–70 per cent of their original infectivity when held for 3 hours at 0°. Willison and Weintraub (7) also have reported that some isolates lost infectivity in extracts at 0°, whereas others lost little or no infectivity in several hours at this temperature.

Quantitative measurements of rate of infectivity loss in diluted extracts agreed well with data on maximum time required for loss of all infectivity for cucumbers. Virus A lost infectivity more rapidly over a 3-hour period than did the other viruses, and it lost all infectivity for cucumbers more quickly than did the others. Virus B was the most stable of the 4 viruses in both kinds of tests, except that differences in relative stabilities of viruses B and E were evident only in the quantitative tests.

Several trials with virus A demonstrated that extracts held at air temperatures of −5° to −10° until frozen were not inactivated. Extracts kept frozen for 3 hours, however, lost most of their infectivity. Extracts frozen rapidly by immersing tubes in an ice-brine mixture at −25° were inactivated immediately.

TABLE 2.—*Rate of loss of infectivity of Prunus viruses in undiluted cucurbit extracts that were allowed to stand for various periods, then diluted 1:80 with buffer and used immediately for inoculation of local-lesion hosts*

Seconds	No. lesions with indicated virus[a]			
	A	B	E	G
0[b]	188	58	92	170
10	45	46	49	82
30	29	34	43	68
90	5	12	23	13

[a] Values are averages of total lesions on 3 half-leaves in each of 3 trials.
[b] Tissue ground in buffer.

TABLE 3.—*Rate of loss of infectivity of Prunus viruses when infected cucurbit tissue was ground in 0.03 M phosphate buffer (1:80) and the extracts allowed to stand for various periods at 0° or 24°C*

Temperature	Hours of standing	No. lesions with indicated virus[a]			
		A	B	E	G
24°	0	216	244	207	109
	1	12	164	62	77
	2	6	128	39	40
	3	3	116	14	4
0°	0	210	192	86	120
	1	166	193	59	81
	2	122	183	43	58
	3	80	186	24	39

[a] Values are averages of total lesions on 3 half-leaves in each of 3 trials. Buffer at pH 8.0 was used for viruses A, E, and G and at pH 8.7 for virus B.

All 4 viruses in intact tissue withstood rapid freezing at temperatures of solid CO_2 or of liquid air. In tissue frozen more slowly by exposure to air at −5° to −10°, however, the viruses were inactivated.

Effect of various chemicals on virus stability.—It has been shown for a number of viruses that the presence of a reducing agent in extracts prolongs infectivity. Trials over a 3-hour period were made with viruses A, B, E, and G in extracts containing 0.03 M phosphate buffer and 0.01 M cysteine hydrochloride. This reducing agent did not affect the rate of loss of infectivity of any of the viruses (Table 4). A few other reducing agents, including ascorbic acid, electrolytically reduced iron powder, and Na_2SO_3 were tested; none of these prevented the rapid loss of infectivity.

On the supposition that enzymes might be involved in this rapid loss of infectivity, the effect of NaCN on the rate of inactivation was determined. In tests with 0.001 M NaCN in 0.03 M phosphate buffer, viruses B and E lost less infectivity than in buffer alone (Table 4). Viruses A and G, however, apparently were unaffected by the presence of cyanide. With all 4 viruses, higher concentrations of cyanide markedly reduced infectivity almost immediately.

The fact that 2 of the viruses were partially stabilized by cyanide suggested that there might be more than 1 factor operating to reduce infectivity. Accordingly, combinations of NaCN and cysteine hydrochloride were tried. When infected tissue was ground in 0.03 M phosphate buffer containing 0.01 M cysteine hydrochloride and 0.001 M NaCN, virus B appeared to be completely stable over the 3-hour period tested (Table 4). The other 3 viruses, however, lost infectivity as rapidly as in buffer alone. The partially stabilizing effect of cyanide on virus E was not evident in the presence of cysteine hydrochloride.

TABLE 4.—*Relative stability of 4 Prunus viruses at 24°C in extracts containing various added chemicals*

Additions to 0.03 M phosphate buffer[a]	Virus	Average no. lesions on plants inoculated after indicated no. of hours[b]			
		0	1	2	3
0.01 M Cysteine hydrochloride	A	92	35	15	4
	B	223	132	105	57
	E	70	31	17	12
	G	307	120	68	8
0.001 M NaCN	A	106	36	14	5
	B	139	115	106	83
	E	132	141	116	84
	G	44	4	2	1
0.01 M Cysteine hydrochloride and 0.001 M NaCN	A	61	35	15	7
	B	66	65	64	67
	E	41	25	17	4
	G	89	65	12	2
0.01 M Sodium oxalate	A	165	25	4	0
	B	206	122	62	32
	E	83	14	4	4
	G	252	68	7	5
0.01 M Sodium oxalate and 0.01 M cysteine hydrochloride	A	197	149	48	13
	B	136	114	138	104
	E	108	115	25	5
	G	68	60	24	4

[a] Buffer used for viruses A, E, and G was at pH 8.0; that for virus B was at pH 8.7.

[b] Values are averages of total lesions on 3 half-leaves in each of 3 trials.

The presence of divalent cations in extracts markedly inhibits infection by rose mosaic virus (2), which is similar in many of its properties to the 4 Prunus viruses used in this investigation. With each of the Prunus viruses, it was found that $CaCl_2$ concentrations as low as 0.001 M caused some reduction in infectivity, whereas 0.01 M caused a marked reduction. Similar results were obtained in a small number of trials with the chlorides of barium, magnesium, strontium, cadmium, and cobalt. That this inhibition of infectivity was not permanent was shown by the increase in infectivity when extracts prepared in 0.01 M $CaCl_2$ were diluted 10-fold with water. Complete reactivation, however, was not obtained by precipitating the calcium with phosphate added as buffer to virus extracts in 0.01 M $CaCl_2$. Much more reactivation was obtained by adding sufficient sodium oxalate to precipitate the calcium. Possibly enough calcium remained soluble in the presence of phosphate to reduce infectivity. Sodium ethylenediamine tetraacetate (Sequesterene) at concentrations above 0.001 M inhibited infection by all viruses and was not effective in reactivating virus in 0.01 M $CaCl_2$ extracts.

The efficiency of sodium oxalate in reactivating viruses in a mixture with $CaCl_2$ suggested its use to increase infectivity and to stabilize the viruses in cucumber extracts. At 0.03 M, however, sodium oxalate markedly decreased infectivity of viruses A, E, and G, as compared to the same concentration of phosphate buffer. In a mixture of 0.02 M phosphate buffer and 0.01 M sodium oxalate, this inhibitory effect was only slight. The infectivity of virus B was increased by sodium oxalate, the numbers of lesions produced by 0.03 M sodium oxalate extracts being nearly double the number produced by extracts in 0.03 M phosphate buffer.

The stability of the 4 viruses was not increased by the presence of 0.01 M sodium oxalate in 0.02 M phosphate buffer (Table 4). In fact, the viruses lost infectivity somewhat more rapidly in extracts containing oxalate than they did in buffer alone. Combinations of oxalate and cyanide also failed to stabilize the viruses. A combination of 0.01 M sodium oxalate and 0.01 M cysteine hydrochloride in phosphate buffer, however, resulted in extracts that lost very little infectivity during the first hour. Most, or all, of the infectivity of virus B was retained for the remainder of the 3-hour period (Table 4), but the other viruses lost infectivity rapidly after the end of the first hour. It seems possible that the failure of the combination of oxalate and cysteine hydrochloride to maintain infectivity over a longer period was due

to a reaction between these 2 compounds. This, however, does not explain why the combination was effective in maintaining the infectivity of virus B.

DISCUSSION.—As a group, the 4 Prunus viruses investigated showed some similar properties that are unusual. A low infectivity in 0.1 M buffer indicates an unusual sensitivity to electrolyte concentrations. A rapid loss of infectivity in undiluted extracts was typical of all the viruses, but this characteristic has also been noted for rose mosaic virus (2) as well as other viruses. Unlike the viruses of tomato spotted wilt or tobacco streak, the presence of reducing agents in extracts was without effect on the stability of viruses A, B, E, and G.

Although viruses A, B, E, and G are similar in being very unstable in extracts, there are differences that serve to distinguish some of them. In particular, the properties of virus B in nearly every test were different from those of the other 3 viruses. The unusually low thermal inactivation temperature of 44° found for virus B suggested that it might be even less stable *in vitro* than the other 3 viruses. This was not borne out, however, by the results of the aging tests, which indicated a greater stability at both 0° and 24° for virus B than for the other viruses. The properties of viruses A, E, and G seemed sufficiently different from those of virus B to warrant the conclusion that virus B is a distinct virus.

The properties of virus E differed in some respects from those of viruses A or G. Virus E was somewhat more stable than viruses A and G in maximum longevity trials, and its stability was increased in the presence of NaCN, which was without effect on viruses A or G. These differences suggest that virus E also is distinct. The differentiation, however, is less sharp than with virus B, and the possible relationship with viruses A or G is accordingly in doubt.

Differences in properties between viruses A and G were only minor. In this connection it may be important to note that virus A was isolated originally from a sour cherry tree with recurrent necrotic ringspot, whereas virus G was isolated from a tree with latent necrotic ringspot. Evidence presented by Moore and Slack (6) indicates a strain relationship between the viruses causing these diseases. The data on properties of viruses A and G are consistent with the supposition that these isolates may be related strains.

The fact that combinations of cyanide or oxalate with cysteine hydrochloride were effective in stabilizing virus B, whereas the components separately were ineffective, indicates that at least 2 processes occur, either of which is sufficient to cause inactivation of virus B. It seems probable that cyanide acts by inhibiting the activity of some enzyme that causes the virus to lose infectivity, either directly or indirectly. Since methods effective with virus B failed to stabilize viruses A, E, and G, it is evident that they are sensitive to a factor, which is present or produced in extracts, that does not affect virus B.

The marked sensitivity of all the viruses to calcium ions and the temporary stabilizing effect of oxalate and cysteine hydrochloride on viruses A, E, and G suggest that divalent metallic ions are somehow involved in loss of infectivity. Although all viruses appeared to be equally sensitive to added calcium, the infectivity of virus B was increased by oxalate, whereas the infectivities of viruses A, E, and G were decreased. It thus may be that oxalate affects virus B infectivity because of some property other than the ability to remove calcium from solution.

Use of low buffer concentrations, use of an optimum pH, and the rapid drying of inoculated leaves had additive effects, each treatment increasing infection alone or in combination with others. Nevertheless, it is evident that until methods are found for stabilizing viruses A, E, and G, procedures involving these viruses must be carried out rapidly if a reasonable degree of infectivity is to be retained.

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FUSION BETWEEN SECONDARY SPORIDIA IN CULTURE IS A VALID INDEX OF SEX COMPATIBILITY IN TILLETIA CARIES [1]

C. S. Holton and E. L. Kendrick [2]

SUMMARY

Results from inoculation studies provided evidence that fusion between secondary sporidia in culture is a valid index of compatibility between monosporidial lines of *Tilletia caries*. All pairs of monosporidial lines in which there was fusion between secondary sporidia in culture also produced infection on the host.

Type of culture growth and method of inoculation are important considerations affecting host infection by compatible pairs of monosporidial lines. Wound inoculation may be essential to infection when the growth type of either one or both lines of a compatible pair is mycelial.

Fusion between paired secondary sporidia in culture recently was suggested as a possible index of sex compatibility in certain species of *Tilletia*.[3] By this criterion, a simple plus (+) and minus (−) relationship among complete sets of monosporidial lines from the same and from different spores was indicated for *Tilletia caries* and *T. foetida*. This suggested possible index of compatibility between monosporidial lines, however, was subject to confirmation by proof of the infective capacity of combined pairs. The results of inoculation studies made for that purpose are presented here.

MATERIALS AND METHODS.—Monosporidial lines from Races T-9 and T-16 of *Tilletia caries* were used.[4] These lines were obtained by isolating the primary sporidia from promycelia of germinating spores and transferring them to nutrient agar.[5] Each monosporidial line was pedigreed as to spore number and sporidium, the sporidia being numbered at random. Thus, 280-1 is monosporidial line number 1 from spore 280, etc. A complete set of lines represents all primary sporidia produced by 1 spore.

Race T-9 was represented by 2 complete sets of monosporidial lines, 8 from spore 280 and 11 from spore 281. There was 1 complete set of 9 lines from spore 285 of Race T-16. Compatible pairs of lines, as indicated by fusion of secondary sporidia in culture, were determined by mating 5 pairs of sporidia of each pair of line combinations within each set and between the 2 sets from Race T-9.

For pathogenicity studies, mixed cultures of all compatible pairs were obtained by growing them together on nutrient agar slants in glass vials. These were used to inoculate seedlings of the susceptible Red Bobs spring wheat. The inoculations were made either by the spore shower method[6] or by inserting the inoculum into wounds of 2- to 3-day-old seedlings.[7] Following incubation at 10°C for 3 weeks, the inoculated seedlings were transplanted and grown to maturity in the greenhouse.

The inoculation was repeated when a compatible pair of monosporidial lines, as indicated by sporidial fusion, failed to produce infection. In those lines that produced mycelium instead of sporidia, the pairs were mixed by chopping, and the inoculum was applied through wounds in the seedlings.

In many previous experiments,[7, 8] no infection was obtained from inoculations with single lines or with pairs of noncompatible lines. For this reason, such inoculations were not even made in these recent experiments.

RESULTS.—The results from sporidial matings in culture and from host inoculation studies with 2 complete sets of monosporidial lines of Race T-9 are presented in Table 1. Compatibility as determined by sporidial fusion in culture was confirmed by host infection, and it was in a simple plus (+) and minus (−) relationship. This was true with all pairs of monosporidial lines, both within and between the groups from spores 280 and 281. The plus (+) and minus (−) lines from spore 280 were in an even (4 to 4) ratio whereas in those from spore 281 the ratio was uneven (7 to 4).

The results obtained with a complete set of 9 lines from spore 285 of Race T-16 likewise confirmed the validity of sporidial fusion in culture as an index of compatibility factors in this species. This set of lines was divided as evenly as possible (ratio of 5 to 4) into a plus (+) and minus (−) relationship, and

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TABLE 1.—*Compatibility reactions between all possible pairs of monosporidial lines from 2 spores of Race T-9, as indicated by sporidial fusion and host infection*[a]

Spore	Lines	Reaction between plus and minus groups of indicated monosporidial lines from 2 spores			
		Spore 280		Spore 281	
		+ Group (1, 2, 3, 6)	− Group (4, 5, 7, 8)	+ Group (1, 2, 4, 5, 7, 8, 11)	− Group (3, 6, 9, 10)
280	1, 2, 3, 6	−	++	−	++
	4, 5, 7, 8	++	−	++	−
281	1, 2, 4, 5, 7, 8, 11	−	++	−	++
	3, 6, 9, 10	++	−	++	−

[a] ++ Denotes sporidial fusion in culture and host infection; − denotes lack of sporidial fusion in culture. No inoculations were made with the latter.

all compatible pairs produced infection of the host.

The method of inoculation can be an important factor in demonstrating compatibility of pairs of monosporidial lines through host infection. Some of the compatible combinations used in this study failed to infect the host in the first inoculation test. The inoculations with these pairs were repeated (in some cases 4 times) until infection was obtained. The growth type of the monosporidial lines in culture also was important. Those lines that failed to infect in the first inoculation produced sporidia only sparsely or not at all and consequently were not adapted to inoculation by the spore-shower method. In such cases, infection was obtained only when the inoculum was inserted into the young seedlings through wounds.

The validity of sporidial fusion in culture as an index of compatibility is further supported by the results from numerous single inoculations of a miscellaneous nature. In one such series, 55 of 58 pairs of lines produced infection from the first inoculation. These lines represented 11 spores of Race T-16. In another series of inoculations with 22 pairs of lines from 4 spores of Race T-10, infection was produced by 15 pairs. In still another group of inoculations, infection was obtained with all 17 miscellaneous matings between compatible pairs. Thus a total of 87 positive reactions resulted in the first inoculation with 97 compatible pairs. In all probability the remaining 10 pairs would have produced infection if the inoculation technique had been more effective. The evidence seems conclusive, therefore, that fusion between secondary sporidia in culture is a valid index of complete compatibility between monosporidial lines of *T. caries*.

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PHYTOPATHOLOGICAL NOTES

Oil Inoculation of Wheat with Spores of Puccinia graminis var. tritici.[1] J. B. ROWELL.[2] The feasibility of inoculating field plots of cereals with spores of rust fungi carried in nonphytotoxic oils was indicated by Rowell and Hayden.[3] This method of inoculation was used with outstanding success in 1956 in field plots where uniformity of initial stem rust attacks and consistency in development of a stem rust epidemic during the season were desired for the best possible evaluation of fungicides applied to control rust. A summary of the technique and results may serve as a useful guide to workers interested in the method for inoculating field plots with rust fungi.

A ⅓-acre plot of Marquis wheat was inoculated on the evenings of June 13 and 15 with freshly collected urediospores of Race 15B of *Puccinia graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Guyot carried in Mobilsol 100, an isoparaffinic nonphytotoxic spray oil. The spores were applied with a 2-gal. knapsack sprayer equipped with a low-gallonage Teejet (No. 730039) nozzle, and the entire area was sprayed with inoculum in 30 minutes each night. Spore dosage was based on 2000 spores per tiller, on the assumption that primary infection would be adequate for an epidemic if 10 per cent of the spores were deposited in infection courts and if 0.1 to 1 per cent initiated infection. Approximately 6 g (3×10^9) spores would be required for 1.5×10^6 tillers per acre. Thus, 6 g of spores per gal. per acre were used; haemocytometer counts of this suspension showed 0.8×10^6 spores

[1] Paper No. 3779, Scientific Journal Series, Minnesota Agricultural Experiment Station. Cooperative investigations between the Crops Research Division and the Minnesota Agricultural Experiment Station.

[2] Plant Physiologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

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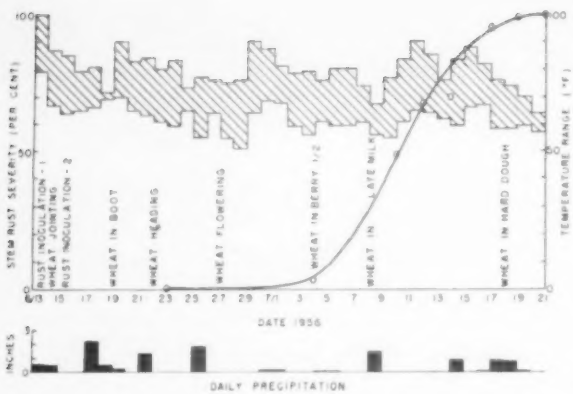

FIG. 1. Development of an epidemic of stem rust initiated by inoculation with urediospores carried in oil on Marquis wheat at St. Paul, Minnesota, in 1956, with pertinent data on plant development, temperature range (cross hatched), and precipitation. Rust severity estimated as percentages of the surfaces of leaf blades and stems covered by rust.

per ml.

The inoculation of June 13 was believed to be ineffective. Weather on that day and during the preceding week was hot and dry, with only traces of dew. Although an overhead irrigation system was turned on from 8 to 10 p.m. after the field was sprayed with inoculum, a brisk, dry, south wind continued to blow until after midnight with a resultant rapid drying of the plants. A rain of 1.02 in. on the morning of June 14 was followed by a heavy dew that night; thus the conditions following the inoculation on the evening of June 15 were favorable for infection. The development of the initial disease and subsequent epidemic is shown in Figure 1. The breakout of rust on June 22 was uniform throughout the field, averaging approximately 10–20 pustules per tiller. No additional infections were apparent until July 3. Thereafter the rust increased very rapidly to over 90 per cent severity in the field within 2 weeks. The rate of increase of disease incidence in the plots followed a typical sigmoid curve similar to that for the growth rate of other microbial populations. Frequent rains and moderate temperatures throughout the period favored repeated infection, and the data illustrate the rate of increase of a rust epidemic that can be attained from an initial uniform inoculation under good conditions. Other years might be less favorable for the development of an artificial rust epidemic.

The success in 1956 of the oil inoculation procedure for inciting an epidemic of stem rust emphasizes the advantages of the method. Spores disperse readily and uniformly in oil, hence a relatively small quantity of spores can be applied rapidly and uniformly to all plants in the field test. The uniform distribution of the subsequent infections eliminates the disadvantages of secondary spread of inocula from rust spreader rows. The simplicity of the operation recommends the method for the inoculation of cereals with spores of rust fungi in large-scale tests.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

A Chromatographic Analysis of the Exudate of Xanthomonas vesicatoria.[1] D. F. CROSSAN AND D. L. LYNCH.[2] The chemical nature of the exudate of bacterial pathogens such as *Xanthomonas vesicatoria* (Doidge) Dows. has long been of interest to plant pathologists because of the probable role the exudate plays in the survival of the organism. It has been reported[3] recently that the viscid exudate of *X. phaseoli* (E. F. Sm.) Dows. consisted of a polysaccharide of high molecular weight.

X. vesicatoria produces a yellow, viscid growth on nutrient and potato-dextrose agars similar to that produced by *X. phaseoli*. Paper chromatography was used as a tool to determine the sugars present in the viscid exudate of *X. vesicatoria*. The exudate was precipitated with acetone and hydrolyzed with 2N H_2SO_4 for 48 hours at 85°C. The material hydrolyzed slowly under these conditions. The viscid growth also was harvested directly with a rubber spatula and hydrolyzed without acetone precipitation for 72 hours at 85°C with 2N H_2SO_4.

The hydrolysates of precipitated and nonprecipitated material were spotted on Whatman No. 1 filter paper and developed 3 times in a mixture of 1-butanol, glacial acetic acid, and water (4:1:5). Partridge's reagent was used as a developer for identification of the sugars present. The following sugars, in order of relative abundance, were identified in both precipitated and nonprecipitated preparations by comparisons with known standards: galactose, glucose, xylose, rhamnose, and a methylated sugar with an R_f value similar to that of 2,3,4,6-tetramethyl-*o*-glucose. There were no qualitative differences between the precipitated and nonprecipitated preparations. In addition, a uronic acid with an R_f value similar to that of glucuronic acid was present.—Department of Plant Pathology and Department of Agronomy, University of Delaware, Newark, Delaware.

A Mature-Plant Type of Wheat Leaf Rust Resistance of Composite Origin.[1] RALPH M. CALDWELL, JOHN F.

[1] Published as Miscellaneous Paper No. 279 with the approval of the Director of the Delaware Agricultural Experiment Station. Contribution No. 98 of the Department of Plant Pathology.

[2] Assistant Research Professor, Department of Plant Pathology, and Assistant Research Professor, Department of Agronomy, respectively.

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[1] Journal Paper No. 1088, Purdue University Agricultural Experiment Station.

SCHAFER, LEROY E. COMPTON, AND FRED L. PATTERSON. The soft red winter wheat variety Dual (C.I. 13083)[2] possesses resistance to leaf rust, caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*, that is currently superior to the resistance of any of its parent varieties. Dual was distributed to growers of Indiana certified seed wheat by the Purdue University Agricultural Experiment Station and the U. S. Department of Agriculture in the fall of 1955. This variety was selected from the last cross of a series of 7 crosses involving the 8 varieties Fultz Selection (C.I. 11512), Hungarian (C.I. 4830), W38-1, Wabash (C.I. 11384), Fairfield (C.I. 12013), Trumbull (C.I. 5657), Hope (C.I. 8178), and Hussar (C.I. 4843). Four of these parent varieties, Fultz Selection, Wabash, Hope, and Hussar, possess certain degrees of resistance to leaf rust. The resistances of 2 of these, Fultz Selection and Wabash, are believed to be the same.

In the Uniform Winter Wheat Rust Nurseries for the years 1950-55, Dual and the immediate parent strain from which it was reselected had an average coefficient of infection by the leaf rust fungus of 9 compared to a coefficient of 51 for Trumbull, the susceptible check variety. Average percentages of infection in the field at Lafayette, Indiana, on Dual and other varieties are shown in Table 1. These tests showed Dual to have a higher level of resistance than the resistant parental types Vigo (representing the Fultz resistance), Hussar, and Hope-Hussar (C.I. 11682), each of which is superior to the susceptible variety Trumbull. Direct comparisons with the spring wheat Hope are not available; however, Hope has been observed to be fully susceptible in the field at Lafayette in recent years. Although showing this high degree of field resistance, Dual is susceptible in the seedling stage to a number of important cultures of the leaf rust pathogen (Table 1). It does not possess the seedling resistance of the Hussar parent. The superior leaf rust resistance of Dual in the mature-plant stage could be either the result of an interaction of the combined parental resistances to produce a higher resistance level or merely an additive effect of these parental resistances to give a broader protection against several differentially virulent races of the pathogen.

This leaf rust resistance of composite origin represented by Dual is the fourth major type of mature-plant leaf rust resistance to be utilized in the eastern soft wheat area. Resistances previously exploited include that derived from Fultz Selection,[3] which was utilized in the varieties Blackhawk[4] and Vigo.[5] This is one of the several components represented in Dual. The other 2 resistances previously used in this region are that from the Brazilian wheats Frondoso and Fronteira[6] (in the varieties, Atlas 66 and Atlas 50,[7] Coastal and Coker 47-27,[8] Anderson and Taylor,[9] and Tayland) and that from Chinese (in Knox[10] and Vermillion).

It is believed that concurrent usage of several dif-

[2] Accession number of the Cereal Crops Section, Crops Research Division, A.R.S., U.S.D.A.

[3] Caldwell, R. M., and L. E. Compton. 1947. Correlated inheritance of resistance to eight races of wheat leaf rust, Puccinia rubigo-vera tritici, powdery mildew, Erysiphe graminis tritici, and glume color. (Abstr.) Phytopathology 37: 4.

[4] Clark, J. A. 1950. Registration of improved wheat varieties, XVIII. Agron. J. 42: 408-409.

[5] Caldwell, R. M., and L. E. Compton. 1947. Vigo—a new disease resistant wheat. Purdue Univ. Agr. Expt. Sta. Bull. 521, 11 p.

[6] Wells, D. G., H. R. Caffey, and S. W. Akhter. 1956. Inheritance of reaction to leaf rust of wheat caused by Puccinia triticina Eriks. in a cross involving a Fronteira derivative. Agron. J. 48: 530-531.

[7] Middleton, G. K., and T. T. Hebert. 1949. Atlas wheat. N. Carolina State Coll. Agr. Expt. Sta. Spec. Cir. 8, 8 p.

[8] Bayles, B. B., and J. A. Clark. 1954. Classification of wheat varieties grown in the United States in 1949. U. S. Dept. Agr. Tech. Bull. 1083, 173 p.

[9] Paden, W. R., and E. B. Eskew. 1953. Anderson and Taylor, two new wheat varieties. S. Carolina State Coll. Agr. Expt. Sta. Cir. 92, 8 p.

[10] Caldwell, R. M., L. E. Compton, J. F. Schafer, and F. L. Patterson. 1954. Knox, a new leaf rust resistant and stem rust escaping soft winter wheat. (Abstr.) Phytopathology 44: 483-484.

TABLE 1.—*Infection by Puccinia recondita on wheat varieties in the field and on seedling wheat plants in the greenhouse, Lafayette, Indiana*

Variety	Type of resistance	Average percentage infection in field (1950-55)[a]	Reaction type[b] to indicated race in greenhouse tests			
			5	35	76	89
Dual	Fultz, Hope, Hussar	4	4	4	4	4
Vigo	Fultz	39	4	4	4	4
Hussar[c]	Hussar	55	2	1+	1+	4
Hope-Hussar[c]	Hope, Hussar	49	2	1	1—	4
Trumbull	None	90	4	4	4	4
Knox	Chinese	trace	4	4	4	4
Purdue Selection (C.I. 12993)	Frondoso	trace	4	4	4	4

[a] Artificially induced epidemics involving Races 5, 9, 15, 31, 35, 76, 89, and 104 (1–6 years each).
[b] Reaction types were classified on the conventional scale, 0 (immune) to 4 (fully susceptible).
[c] Field data for 1950 were obtained from Vincennes, Indiana.

ferent types of leaf rust resistance such as the 4 listed above offers greater over-all protection from leaf rust damage than does the use of only 1 type, even one at a level superior to that of the others involved. The basis for this proposal is that intervarietal diversification of resistance in a wheat-producing area should protect against excessive losses due to the development of a population of the rust pathogen virulent to varieties possessing any single type of resistance. It also allows for geographic diversification of resistance as has occurred in the eastern soft wheat area, where the Fultz resistance is utilized in the northern part and the Frondoso-Fronteira resistance in the south. An illustration of the value of geographic diversity is the effectiveness of the leaf rust resistance of Vigo wheat in Indiana for the last 10 years. Although known to be moderately susceptible to a number of collections of *P. recondita*, Vigo has maintained excellent protection from damage, since its release in 1946, in years of severe infection of susceptible varieties. This effectiveness is attributed to the limited distribution of this Fultz type of resistance. No varieties carrying the Fultz resistance have been distributed in the southern states. There has been very little overwintering of the leaf rust fungus in Indiana during this period, and races specifically virulent to Vigo have not increased in quantity in the source areas of wind-borne spores. In occasional Vigo fields where *P. recondita* has overwintered, this variety has shown markedly greater susceptibility to the races present.

The leaf rust resistance reported here for Dual is combined in this variety with resistance to the Hessian fly (*Phytophaga destructor* Say[11]), resistance to soil-borne wheat mosaic virus, high yielding capacity, winter hardiness, stiff straw, and superior soft wheat milling and baking qualities.—Purdue University Agricultural Experiment Station and Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Lafayette, Indiana.

[11] Caldwell, R. M., and L. E. Compton. 1939. Wheat breeding for the combined resistance to disease and hessian fly. Purdue Univ. Agr. Expt. Sta. Ann. Rept. 51 (1938): 41-42.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

BRAUN, H., AND E. RIEHM. 1957. **Krankheiten und Schädlinge der kulturflanzen und ihre Bekämpfung.** Paul Parey, Berlin. 368 p. 346 Illus. Price DM 29.80.

The volume includes both diseases and insect pests. The first 30 pages are general discussions on symptoms, cause, life history, and control. The remainder of the book is composed of 22 discussions, dealing with individual crops or groups of crops, and a rather complete index. The subjects are cereal, potato, beet, carrot, clover, lupine, alfalfa, serradella, bean and pea, crucifer, tomato, onion, asparagus, cucurbit, rape, flax, poppy, tobacco, hop, fruit, grape, and general diseases and pests. The limited space and the wide range of crops permit only short paragraphs to half pages for most of the diseases. Only a few, like cereal rust and apple scab, are dealt with in more detail. The illustrations, quality of paper, and the printing are excellent.

CUSHING, J. E., AND D. H. CAMPBELL. **Principles of immunology.** McGraw-Hill Book Co., New York. 344 p. Price $6.30.

Designed primarily for advanced undergraduates and for graduate students, this book introduces the basic principles and concepts of immunology and immunochemistry. The first part is a general survey of aspects of the physical sciences relevant to this field and of terms and concepts. The second part deals with biological problems in which immunological problems and concepts play an important role, and the third part concerns the nature of antigens, antibodies, and their reactions. The general reactions found useful in plant serology are presented in some detail, and immunity in plants is treated briefly.